637.127.6:576.809.3

# 298. THE OCCURRENCE OF SLOW-REDUCING COLIFORM ORGANISMS IN MILK

BY C. S. MORRIS, *Seale Hayne Agricultural College, Newton Abbot, Devon*

During 1937, when the methylene-blue reduction test was adopted as the official test for the examination of graded raw milks, it was noted that 8–10 % of the official accredited milk samples received for examination at this laboratory did not reduce methylene blue in 7 or 8 hr., and yet the presumptive coliform test showed that coliform organisms were present in 1/1000 ml.

Since Wilson (1) found the coliform group to be quick reducers of methylene blue it was felt that further information should be obtained by testing in milk the reducing properties of these apparently slow reducing strains. Since 1938 the coliform organisms from any milk sample which had a reduction time of over 6 hr., and which contained coliform organisms in 1/1000 ml. were therefore isolated in pure culture and tested for their reducing properties when inoculated into 'sterile' raw whole milk.

## THE TECHNIQUE ADOPTED FOR OBTAINING AND TYPING THE CULTURES

Pure cultures of the organisms were obtained by plating dilutions made from the positive MacConkey broth tubes on MacConkey agar.

Differential tests were applied to each culture so isolated. The results reported in Table 1 refer to organisms typed in this manner, which appear to be irregular types of coliform organisms.

Table 1. *Some results obtained by differential tests on ten cultures*

| Culture no. | Citrate | Indol | Voges-Proskauer | MacConkey at 44° C. | Methyl red | Gelatin |
|---|---|---|---|---|---|---|
| 1 | – | – | – | – | + | – |
| 2 | – | . | – | – | + | – |
| 3 | – | – | – | – | + | – |
| 4 | – | – | – | – | – | – |
| 5 | – | – | – | – | + | – |
| 6 | – | – | . | – | + | – |
| 7 | – | – | – | – | + | – |
| 8 | – | . | – | – | + | – |
| 9 | – | – | – | – | + | – |
| 10 | – | – | – | – | + | – |

It will be noted that most of the cultures were citrate negative, but showed no growth or gas production when cultivated in MacConkey's broth at 44° C.

## THE TECHNIQUE FOR TESTING THE REDUCING PROPERTIES OF THE ORGANISMS WHEN INOCULATED INTO RAW WHOLE MILK

An 18–24 hr. old agar plate culture of the organism to be tested was washed off with 10 ml. of sterile tap water, and centrifuged for 15 min. at about 3000 r.p.m. The supernatant fluid was then discarded and the deposit suspended in sterile tap water to match an opacity tube reading of 1000 million organisms per ml.

Dilutions of this suspension were then made, and a quantity of the diluted suspension added to fresh raw milk (having a plate count of less than 1000 per ml. and coliform organisms absent in 1 ml.), so as to give an inoculation of approximately 200,000 per ml. of the milk. Methylene-blue reduction tests, plate counts and coliform tests were then carried out on the inoculated milk.

The results obtained with three strains of slow reducing coliform organisms are given in Table 2. These may be compared with the results for normal strains which are shown in Table 3.

Table 2. *Results of reduction tests, plate counts and coliform tests carried out on slow-reducing strains of coliform organisms inoculated into fresh raw milk*

| Organisms | Plate count at time of carrying out the methylene-blue test per ml. | Presumptive coliform test present in ml. | Methylene-blue reduction time hr. |
|---|---|---|---|
| No. 1 | 414,000 | 1/10,000 | 9 |
| | 249,000 | 1/10,000 | 9 |
| | 439,000 | 1/10,000 | 10 |
| | 745,000 | 1/10,000 | 8½ |
| | 323,000 | 1/10,000 | 9½ |
| | 249,000 | 1/10,000 | 9 |
| | 488,000 | 1/10,000 | 10½ |
| | 206,000 | 1/10,000 | 9½ |
| No. 2 | 300,000 | 1/10,000 | 9 |
| | 161,000 | 1/10,000 | 10 |
| | 159,000 | 1/10,000 | 8½ |
| | 300,000 | 1/10,000 | 9 |
| | 618,000 | 1/10,000 | 8½ |
| No. 3 | 280,000 | 1/10,000 | 8½ |
| | 360,000 | 1/10,000 | 7½ |
| | 180,000 | 1/10,000 | 8 |

Table 3. *Results of reduction tests, plate counts and coliform tests carried out on normal reducing strains of coliform organisms isolated from accredited milk samples and inoculated into fresh raw milk*

| Organisms | Plate count at time of carrying out the methylene-blue test per ml. | Presumptive coliform test present in ml. | Methylene-blue reduction time hr. |
|---|---|---|---|
| No. 1 | 372,000 | 1/10,000 | 4½ |
| | 257,000 | 1/10,000 | 4½ |
| | 393,000 | 1/10,000 | 4½ |
| No. 2 | 122,000 | 1/10,000 | 5 |
| | 372,000 | 1/10,000 | 4½ |
| | 393,000 | 1/10,000 | 4½ |
| No. 3 | 150,000 | 1/10,000 | 5 |
| | 478,000 | 1/10,000 | 4½ |
| | 588,000 | 1/10,000 | 4½ |

It was thought that the slow reduction of the methylene blue might be due to the presence of a specific growth-inhibitory factor in the milk such as Hobbs (2) has described. Plate counts on MacConkey agar were therefore carried out on the methylene-blue tubes every 2 hr., with the results shown in Table 4.

Table 4

| | Culture no. 4 per ml. | Culture no. 5 per ml. | Culture no. 6 per ml. |
|---|---|---|---|
| Plate count at time of inoculation | 460,000 | 420,000 | 460,000 |
| Plate count after incubation at 37° C. for 2 hr. | 280,000 | 280,000 | 580,000 |
| Plate count after incubation at 37° C. for 4 hr. | 4,500 | 1,200 | 450,000 |
| Plate count after incubation at 37° C. for 6 hr. | 2,600 | 800 | 86,000 |

Thirty-five cultures have been tested in this way, and have given similar results. The slow reduction of methylene blue by these cultures would thus appear to be due to two factors: (*a*) the presence of an actual bactericidal substance in the milk specific for these organisms, and (*b*) the presence of a growth inhibitory factor such as has been described by Hobbs.

To find if the growth inhibitory factor was destroyed by heat, as demonstrated by Hobbs(3), milk which had been heated to 70° C. for 1 hr. was inoculated, a methylene-blue test was carried out at the time of inoculation, and plate counts on MacConkey's agar were made every 2 hr. during incubation. The results are shown in Table 5.

Table 5. *Plate counts and reduction tests after inoculation with slow cultures*

| | Raw milk | | Heated milk Milk heated to 70° C. for 1 hr. | |
|---|---|---|---|---|
| | Plate count per ml. | Methylene-blue reduction time hr. | Plate count per ml. | Methylene-blue reduction time hr. |
| Plate count at time of inoculation | 426,000 | 7½ | 490,000 | 8½ |
| Plate count after incubation at 37° C. for 2 hr. | 3,200 | 7½ | 2,500,000 | 8½ |
| Plate count after incubation at 37° C. for 4 hr. | 1,200 | 7½ | 15,000,000 | 8½ |
| Plate count after incubation at 37° C. for 6 hr. | 80 | 7½ | 200,000,000 | 8½ |

Twenty-five cultures were tested out with similar results. Control tests on the raw uninoculated milk showed that this never had a bacterial count of over 2000 organisms per ml., or a methylene-blue reduction time of under 7½ hr.

Control experiments were also carried out to ascertain the rate of multiplication of normal coliform organisms in both raw and heated milks, the results being shown in Table 6.

Table 6. *Plate counts and reduction tests after inoculation with normal cultures*

| | Raw milk | | Heated milk Milk heated to 70° C. for 1 hr. | |
|---|---|---|---|---|
| | Plate count per ml. | Methylene-blue reduction time hr. | Plate count per ml. | Methylene-blue reduction time hr. |
| Plate count at time of inoculation | 830,000 | 3 | 950,000 | 6½ |
| Plate count after incubation at 37° C. for 2 hr. | 1,600,000 | 2 | 819 million | 5 |
| Plate count after incubation at 37° C. for 4 hr. | 2,610 million | 1 | 58,000 million | 3 |
| Plate count after incubation at 37° C. for 6 hr. | 411,000 million | ½ | 3,424,000 million | 1 |

From the above results it would seem that both the bactericidal substance and the growth-inhibitory factor present in raw milk are destroyed by exposing milk to a temperature of 70° C. for 1 hr.

When the growth-inhibitory factor is destroyed by heat the organisms appear to multiply rapidly, but in spite of this rapid growth they do not reduce methylene blue. It was

thought that the marked decrease in plate count might be due to an agglutinating factor in the raw milk. Milk was therefore inoculated with approximately 10 million organisms per ml., and incubated in a water-bath at 37° C. Breed smears were made at ½ hr. intervals, and examined for bacterial clumping. No clumping could be demonstrated, but there was a decided decrease in the number of bacterial cells per field.

The possibility that such a small inoculation of organisms into the milk was responsible for the bactericidal effect noted with some cultures, and the long lag phase of growth with others, was considered, and raw 'sterile' milk was therefore inoculated with approximately 1,000,000 organisms per ml. with the results shown in Table 7.

Table 7. *Effect of the size of the inoculum*

| Inoculation count per ml. | Colony count on MacConkey's agar after incubation at 37° C. for 6 hr. | Methylene-blue reduction time hr. | Percentage decrease |
|---|---|---|---|
| 1,600,000 | 9,000 | 7½ | 99 |
| 1,081,000 | 1,000 | 6½ | 99 |
| 160,000,000 | 200,000 | 6½ | 99·78 |
| 102,000,000 | 600,000 | — | 99·42 |
| 1,800,000 | 1,400,000 | 7 | — |
| 160,000,000 | 1,900,000 | 6½ | — |

From these results it would seem that the size of inoculum does not affect either the bactericidal action, or the long lag phase of growth.

It was felt that the bactericidal effect might be due to a bacteriophage present in the raw milk. Milk in which the organisms had been incubated at 37° C. for 6 hr. was put through a Seitz filter and the filtrate added to a young nutrient broth culture of the organism. A mixture of the culture and the filtrate was also streaked sparingly over the surface of a nutrient agar plate. These broth cultures and plates were then incubated at 37° C. for 24 hr. and examined for lysis and 'plaques' respectively. The results were negative.

## Summary

Milk samples may frequently contain coliform organisms which give very slow reduction of methylene blue at 37° C.

This slow reduction seems to be due to two factors present in raw milk, both of which are destroyed by heating milk to 70° C. for 1 hr. These factors are (*a*) a specific bactericidal substance, and (*b*) a growth-inhibitory factor.

All the cultures examined appeared from differential tests to be intermediate types of coliform organisms. All were citrate negative and failed to produce acid or gas when grown in MacConkey's broth at 44° C. This tends to confirm the findings of Taylor(4) that the use of certain tests for the differentiation of coliform bacteria is not justified.

## REFERENCES

(1) Wilson (1935). Bacteriological Grading of Milk. *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 206, p. 269.
(2) Hobbs (1939). *J. Dairy Res.* **1**, 49.
(3) Hobbs (1939). *J. Dairy Res.* **1**, 50.
(4) Taylor (1941). *Proc. Soc. agric. Bact.* (Abstracts), p. 15.

(*MS. received for publication* 10 *March* 1942)

637.333.1:576.809.6

# 299. FURTHER STUDIES ON BACTERIOPHAGE IN RELATION TO CHEDDAR CHEESE-MAKING*

By C. K. JOHNS†

Early in 1941 Johns & Katznelson (1) described the sudden arrest of acid development in several experimental vats of Cheddar cheese caused by a polyvalent streptococcal phage. Although the starter in use (no. 21) consisted of a mixture of strains, the stoppage was as abrupt as in the cases reported where single-strain starters have been employed (2). When a fresh starter composed of different bacterial strains (no. 2) was substituted, the trouble ceased.

Starter no. 2 was used throughout the remainder of October and November 1940 and for a single vat in December, after which no further cheese was made until 16 April 1941. Believing that the phage had been destroyed by measures taken to sterilize all cheese-making equipment after the first outbreak, the cheese-maker reverted to starter no. 21, which he preferred to no. 2. On the second day of making, one vat showed very slow acid development, with almost complete absence of starter organisms in the whey. Phage apparently identical with that encountered in October was readily demonstrated in a Seitz EK filtrate from the whey. An entirely different starter, no. 43, was then substituted and no further trouble was encountered.

## Source of phage

Since the starter itself again appeared to be entirely free from phage, it seemed most probable that the phage had either carried over on the equipment from the October outbreak, or else had come from the milk supply. It was of course too late to determine whether or not phage had been carried over on the equipment. However, it seemed unlikely that such would be the case in view of the drastic sterilizing treatment employed. Furthermore, cheese had been made with starter no. 2 on 14 days in October, November and December. Attention was therefore directed mainly to the milk supply.

The milk used at the time of the April outbreak came from the Central Experimental Farm herd. Pail samples were obtained from each of fifty-two cows, but no phage could be demonstrated when full test-tubes of milk were seeded with a dilute suspension of starter no. 21, and repeated subcultures made into litmus milk tubes also seeded with this starter. Nevertheless, between 20 and 26 April, daily samplings of approximately 100 ml. volume taken from the pasteurizer vat before heating, comprising the combined night's and morning's milkings, were with one exception positive for phage! Samples of the same milk taken *after* pasteurization were negative except for the sample taken on 24 April. Samples were also taken directly from the 8 gal. shipping cans in which the milk is transported to the Farm Dairy; a single can sampled on 19 April was positive, as were four out of seven from the evening's milk of 25 April. The remainder of the sixty-four can samples examined was negative. None of eighteen samples taken at the dump vat, nor of the first and last milk pumped over into the pasteurizer, gave a positive result. Phage could not be demonstrated in the air of the receiving and pasteurizing rooms, the milk

* Contribution No. 153 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Canada.

† Associate Bacteriologist.

room at the dairy barn, nor in the laboratory. Negative results were obtained when the hands of the dairy employees were rinsed in sterile milk, but positive results were obtained from the author's hands on two out of four trials, and once from his saliva out of seven trials. However, neither of these can be regarded as the original source, since the author had had no contact with either starter or cheese room for some time prior to this outbreak.

The concentration of phage in the milk diminished throughout the investigation. After the first week it was never present in sufficient quantity to prevent clotting of the original sample. Its presence was demonstrated only twice by subculturing subsequent to 1 May, while from 19 May on it could not be found, even in pint samples.

### Factors concerned in the demonstration of the presence of phage in milk

During these studies it became evident that at least two factors influenced the ease with which the presence of phage could be demonstrated. The first of these was the size of sample. As previously mentioned, 100 ml. samples from the vat were regularly positive while test-tube samples from individual cows, cans, etc., remained negative. Further evidence was obtained by filling test-tubes from half-pint samples which had been seeded with starter. On two occasions the half-pint sample was positive while the smaller sample remained negative. On four other occasions both sets remained negative.

The second factor which appeared to influence the results was the temperature at which the sample was stored prior to seeding and incubation with the starter. The 100 ml. samples of raw and pasteurized milk taken at the vat were normally held at around 13° C. for approximately 7 hr. before being tested for the presence of phage. Suspecting that this might have something to do with the more frequent demonstration of the presence of phage in such samples, samples were divided, one portion tested at once and the other stored at 10–15° C. for 7–16 hr. before testing. Positive results were obtained much more frequently with the latter. Further confirmatory evidence was obtained by taking triplicate half-pint vat samples of both raw and pasteurized milk; one of each was held at 4° C., one at 13° C. and one at 24° C. for 8 hr. before testing. Out of 12 days' samplings, only one raw (7 May) and one pasteurized (6 May) sample showed the presence of phage; both of these had been held at 4° C. before testing.

These two factors, size of sample and treatment before testing, may help to explain some of the anomalies referred to. Samples of milk from individual cows and from shipping cans were test-tube samples, and almost invariably were seeded with starter and incubated without delay. Vat samples, on the other hand, comprised five or six times as much milk. The fact that no phage was demonstrated in the milk from individual cows or cans, even when the *total* quantity of milk taken in all these individual samples was often equal to, or greater than, the volume of vat milk examined, suggests that the preliminary holding of vat samples at a lower temperature before testing was the more important factor.

### Delayed acid production in commercial factories

Slow acid development for which no apparent cause can be found is not uncommon in cheese-making areas. Since most streptococcal phages are fairly specific in their action (2), and since mixed cultures are commonly employed in eastern Canada, it is to be expected that phage infection would most probably attack one or two strains of lactic streptococci,

causing a lag in acid development rather than an abrupt and complete stoppage such as was encountered in the outbreaks at the Central Experimental Farm. That this type may also occur has recently been proved in the case of two cheese factories some 60 miles west of Ottawa.*

At both of these factories acid developed well in the starter, in the vat milk and in the whey until the cooking temperature (96–100° F.) had been reached. At about this stage the rate of acid production slowed down markedly, the remainder of the manufacturing process was seriously retarded, and the resulting cheese was graded down for weak body, open texture, gas holes and, frequently, for 'fruity flavour'. In order to make a satisfactory grade of cheese both makers had modified their process by setting at a much higher acidity (0·23–0·26 %) and allowing the whey to reach an acidity of at least 0·40 % before dipping the curd.† By the time the curd was piled the whey acidity was about 0·55–0·6 %. This condition had persisted for some 6 weeks prior to the author's visit to these factories on 3 September 1941.

This peculiar behaviour strongly suggested that the dominant strains in the starter were being lysed by phage shortly after cooking temperature was reached, subsequent acid production being the work of resistant strains from the starter or the milk itself. Samples of starter and whey were therefore brought to the laboratory for study. The technique employed in the Central Experimental Farm outbreaks (seeding litmus milk with starter and whey and comparing with milk plus starter) gave no indication of phage activity. Plates were next poured from the starters (both of which contained similar bacterial strains, having been obtained from the same source), and a series of representative colonies fished. Yeast extract-whey broth cultures of these were grown for 16 hr. at 32° C. Four fresh tubes of broth were then seeded from each of these; one was left as control, while each of the others received 0·5 ml. of a Seitz EK filtrate from the whey of the two factories (A and B) and of a third (C) which had had somewhat similar trouble, but was then clear of it. Tubes were incubated at 32° C. with hourly observations for lysis until the 8th hour, then after 13 and 24 hr.

The twenty-seven colonies fished for testing could be separated into at least nine separate strains on the basis of the tests outlined by Yawger & Sherman (5). The whey filtrates from factories A and B both caused strong lysis in three of these strains, but only a slight amount in the remainder. The whey filtrate from factory C, where the trouble had disappeared, failed to cause lysis of any of the strains tested.

While some degree of lysis was noted in each of the nine strains tested, growth in most of the cultures was only slightly inferior to that in the control tube. These more resistant strains, together with the lactic organisms coming from the farms in the milk, apparently provided sufficient acid development at stages subsequent to the lysis of the more susceptible organisms to enable the makers to turn out a fairly good grade of cheese by the modified technique they had developed. This is in sharp contrast with the two outbreaks at the Central Experimental Farm where the quality of the cheese was seriously lowered by the complete destruction of the starter organisms.

Both factories A and B had been obtaining starter cultures from the same source

* Slowness in the vat where mixed cultures were used has since been reported by Meanwell (3) and Harrison & Wolf (4) as being due to phage action.

† The condition of the curd at this stage resembled that of a normal vat with a whey acidity of around 0·28 %, suggesting that the phage was mainly concentrated in the curd, with a consequently slower development of acid there than in the extruded whey.

throughout the season. Investigation revealed that only one culture was being propagated by the agency supplying the cultures so that the starter at both factories contained similar bacterial strains. During the outbreak both makers had repeatedly sent for new cultures from the same source, but these were naturally just as susceptible as the ones they replaced. The substitution of a starter of entirely different bacterial composition promptly eliminated the difficulty with slow working, further confirming the view that phage was the cause of the trouble.

Still further confirmation of the fact that phage was the cause of these slow working vats was obtained a month later from factory B. Upon learning that this factory was again experiencing trouble, a second visit was paid. It was found that the maker had reverted to the original susceptible starter, obtained from a neighbouring factory. A whey sample taken the day after the reintroduction of the susceptible starter was found to contain phage causing complete lysis of the broth culture of one of eleven colonies isolated from the starter in question. Anticipating that this might have been the reason for the recurrence of this trouble a culture of our starter no. 21 was left with the maker. As in the case of starter no. 43, previously left with him, normal acid production followed.

There is no reason to believe that the susceptible starter itself contained phage. This starter had been supplied to several hundred factories in this area during the past season without our hearing of any other trouble from phage, while we have been unable to demonstrate the presence of phage in laboratory tests.

## Discussion

At the time these studies were conducted, we were unable to find any reference in the literature to slow acid development in a commercial factory using a multiple strain starter being due to a phage capable of lysing the dominant strains.

When slow working vats are encountered under circumstances suggestive of phage action, makers should have impressed upon them the necessity of obtaining a fresh starter of entirely different bacterial flora. Had the importance of this been realized in the two instances described, a great deal of loss and trouble could have been avoided.

## Summary

A second outbreak of phage infection with complete cessation of acid production in experimental vats is described. Although occasional positive indications were obtained from the milk supply itself, the original source of the phage was not definitely demonstrated.

The results obtained in testing for phage appeared to be influenced by the size of milk sample examined, and by refrigerated storage for 7–16 hr. before testing.

Trouble with slow working vats in two factories which had continued over a period of 6 weeks was shown to be due to lysis of the dominant strains of the mixed culture of starter organisms by phage.

Attention is called to the need for substitution of a starter of entirely different bacterial strains when such trouble is encountered.

## REFERENCES

(1) Johns & Katznelson (1941). *Canad. J. Res.*, C, **19**, 49.
(2) Whitehead & Hunter (1939). *J. Dairy Res.* **10**, 403.
(3) Meanwell (1941). *Proc. Soc. agric. Bact.* (Abstracts), p. 16.
(4) Harrison & Wolf (1941). *Proc. Soc. agric. Bact.* (Abstracts), p. 19.
(5) Yawger & Sherman (1937). *J. Dairy Sci.* **20**, 205.

(*MS. received for publication* 3 *September* 1942)

637.333.1:576.809.6

# 300. THE INFLUENCE OF ABNORMAL ('NON-ACID') MILK ON CHEESE STARTER CULTURES

By G. J. E. HUNTER and H. R. WHITEHEAD,

*Dairy Research Institute (N.Z.), Palmerston North, New Zealand*

(With 1 Figure)

Failures of starter cultures due to destruction of the lactic streptococci by bacteriophage are very frequent in commercial cheese factories in New Zealand unless special precautions are observed (1,2). Occasionally, however, we have observed instances where a culture failed to clot milk within an 18 hr. incubation period, yet bacteriophage could not be detected in the thin culture. In one case the sterilized milk, inoculated with a pure culture of a strain of *Str. cremoris* (*HP*—the most widely used single strain culture), failed to coagulate in 18 hr. although it thickened in 24 hr., whereas some similar milk under the same conditions inoculated with another strain of *Str. cremoris* ($R_1$) coagulated within the usual period of 18 hr. The absence of a phage for *HP* in the thin culture indicated that the fault probably lay with the milk. Inquiry showed that at this particular time the factory manager, contrary to usual practice, had ceased to select morning's milk for the preparation of starter and that night's milk mainly from one supplier had been used. There was no opportunity at the time for direct proof of the hypothesis that a particular supply of milk caused the trouble, but trials in the laboratory subsequently proved that 'non-acid' milk can cause such trouble and that it affects some cultures more readily than others.

'Non-acid' milk is a colloquial term coined by cheese-makers in New Zealand to designate milk which hinders the development of acid by a normally active starter culture in the cheese vat. Whitehead & Riddet (3) isolated from certain milk supplies a *Streptococcus* which formed, during its growth in milk, an inhibiting substance capable of delaying the growth of normal lactic streptococci subsequently added to the milk in the vat. They showed that the inhibitory substance was not destroyed when the milk was heated to 212° F. for 30 min. Whitehead (4) described in detail the biochemical characteristics of the 'non-acid' streptococci.

The difficulty caused by 'non-acid' milk is usually confined to one or two of the cheese vats in a commercial factory, since in general it is due to the night's milk delivered by one or more suppliers. The starter cultures themselves are unlikely to be affected owing to the fact that it is the general custom to select for starter milk the cleanest morning's milk delivered to the factory. 'Non-acid' milk with a really powerful inhibitory effect is now so rare, however, that in the case cited above the manager was very unlucky to have trouble with his starter even with milk taken at random. The occurrence drew our attention to the possibility that 'thin' starters might sometimes be due to 'non-acid' milk and the following experiments were planned to test the suggestion that normal starter streptococci might vary in their reaction to the inhibitory principle.

### Preparation of artificial 'non-acid' milk and general experimental method

Stock cultures of 'non-acid' organisms were grown at 30° C. in sterilized skim milk for 24 hr. The acidity rise was 0·03–0·06 % expressed as lactic acid. Varying proportions of this milk were then added to normal fresh milk in conical flasks and the mixtures were steamed for 30–45 min. The flasks were sown with $1\frac{1}{2}$ % of various starter cultures and incubated at 20° C. in the usual way.

### Growth of *cremoris* strains *HP* and $R_1$ in 'non-acid' milk

To normal skim milk 10 % 'non-acid' milk was added and the mixture was distributed in two flasks. Control flasks of normal skim milk were set up, and after all the flasks had been steamed and cooled to 22° C. cultures *HP* and $R_1$ were each sown into flasks of normal and 10 % 'non-acid' milk. After incubation at 22° C. for 17 hr. both control flasks were clotted. The flask of $R_1$ in 'non-acid' milk was also clotted, but the flask of *HP* in 'non-acid' milk was still liquid. It began to coagulate $2\frac{1}{2}$ hr. later. The acidities of the cultures expressed as percentage lactic acid at 17 hr. were as follows:

| | Normal milk cultures | 10 % 'non-acid' milk cultures |
|---|---|---|
| *HP* | 0·82 | 0·39 |
| $R_1$ | 0·78 | 0·78 |

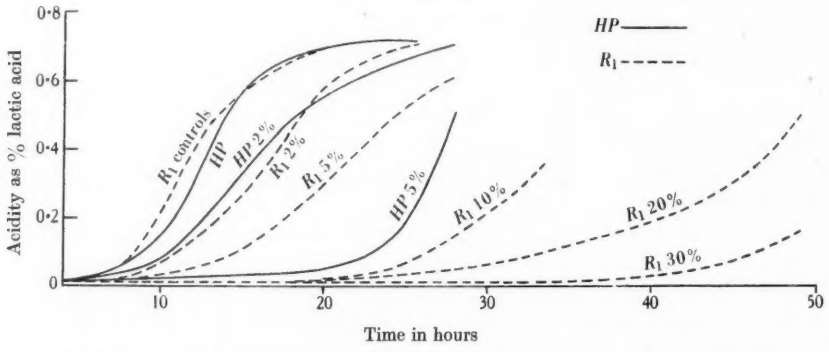


Fig. 1. Acid produced by cultures *HP* and $R_1$ in milk containing various proportions of 'non-acid' milk.

These results show that *HP* is more strongly affected than $R_1$ by the inhibitory substance in 'non-acid' milk. Further experiments showed that the difference between *HP* and $R_1$ in their reaction to the presence of the inhibitory substance is one of degree. Culture $R_1$ is restrained in its growth, but it overcomes the inhibition much more rapidly than does *HP*. The above results reproduce almost exactly the phenomenon which had been encountered in the commercial factory, and it seems most probable that the trouble was in fact due to the accidental use of 'non-acid' milk for preparation of the bulk starter.

The growth of *HP* and $R_1$ in milk containing varying proportions of 'non-acid' milk is shown graphically in Fig. 1. In the experiment from the results of which the graph was drawn 9 ml. quantities of milk containing proportions of 'non-acid' milk ranging from nil to 30 % were steamed in the usual way and then inoculated with 2 drops of

starter culture from a 1 ml. pipette. All the tubes were incubated at 22° C. and the development of acid was followed by titration of representative tubes at intervals. The curves show how *HP* was very much more strongly inhibited at all stages than $R_1$; *HP* formed no acid at all within the period of the experiment in the presence of more than 10 % of 'non-acid' milk. Culture $R_1$, although inhibited quite strongly by the higher proportions of 'non-acid' milk, showed a greater power of recovery. As was to be expected, the precise degree of inhibition of both cultures in different experiments varied according to the batch of 'non-acid' milk used. The amount of 'non-acid' substance formed obviously depends on the precise extent of growth of the 'non-acid' organism and probably upon the strain of organism. Nevertheless, the general trend of the results was the same in all experiments.

### GROWTH OF VARIOUS *CREMORIS* STRAINS AND OF COMMERCIAL STARTER CULTURES IN 'NON-ACID' MILK

The effect of 'non-acid' milk on a series of starter cultures was investigated. Table 1 gives the acidities of cultures after 17 hr. incubation in normal milk and in milk to which 10 % of 'non-acid' milk had been added.

Table 1. *Acidities in starter cultures as percentage lactic acid*

| Type of culture | Culture | Control milk | 10 % 'non-acid' milk | |
|---|---|---|---|---|
| | | | Batch 1 | Batch 2 |
| Pure strains of *Str. cremoris* | *HP* | 0·73 | 0·44 | 0·62 |
| | $R_1$ | 0·76 | 0·72 | 0·74 |
| | $R_6$ | 0·72 | 0·44 | 0·75 |
| | *K* | 0·65 | 0·31 | 0·71 |
| | *RWI* | 0·72 | 0·19 | 0·19 |
| | *FH* | 0·70 | 0·79 | 0·80 |
| Commercial mixed cultures | *E* | 0·80 | 0·30 | 0·33 |
| | *F* | 0·72 | 0·29 | 0·29 |
| | *G* | 0·72 | 0·72 | 0·72 |

The different results obtained with the two batches of 'non-acid' milk illustrate the point already mentioned about the variability in concentration of the inhibitory substances. The amount present when batch 2 was used was not sufficient to restrain *HP* markedly, yet it still affected *RWI* strongly. It is evident that strains of *Str. cremoris* show a whole range of sensitivities to 'non-acid' milk. Experiments in which still more inhibitory substance was present in the culture milk showed that of all the cultures tested *FH* was the most hardy in the sense that it produced acid in milk in which all the other cultures were completely checked.

### DISCUSSION

It is evident that to the two common causes for 'thin' starter, bacteriophage action and low temperature of incubation, must be added a third one, viz. the presence in the starter culture milk of a proportion of 'non-acid' milk. The actual sequence of events in cultures where one or other of these three influences is at work is of course somewhat different in each case. Too low a temperature of incubation merely results in a low reproduction rate and hence in a delayed acid production. The presence of inhibitory substance in the milk (so-called 'non-acid' milk) results in a prolonged lag period which varies in different instances according to the proportion of inhibitory substance present and the strains of streptococci present. The sequence of events in the culture is very similar to that which

obtains with a low incubation temperature. Infection with bacteriophage, on the other hand, results in a normal or possibly supranormal reproduction rate at first followed by lysis of the bacteria. Whereas with low temperature or 'non-acid' milk the culture finally coagulates, with bacteriophage attack there is no further acid production after lysis has taken place until resistant forms develop some 24–48 hr. later. It is important to bear in mind the several possible causes for failure of a starter culture to coagulate in a normal time when conducting field trials, otherwise one can easily be misled.

The results of the experiments described in this paper indicate that it is desirable to select fresh morning's milk for the preparation of the starter because even under uncleanly conditions of production, morning's milk is unlikely to be held long enough to permit growth of 'non-acid' streptococci to a sufficient extent to render the milk inhibitory. It is of interest to note that commercial mixed starters as well as single strain cultures vary in their ability to overcome the inhibitory effect.


## Summary

If milk containing the inhibitory substance produced by growth of 'non-acid' streptococci is used for the propagation of starter, it can cause delayed coagulation, simulating a starter failure caused by bacteriophage. 'Non-acid' milk in the starter culture milk affects cultures differently according to the particular strains of streptococci present; some strains are considerably less inhibited than others.


## REFERENCES


(1) Whitehead, H. R. & Hunter, G. J. E. (1939). *J. Dairy Res.* **10**, 120.
(2) Whitehead, H. R. & Hunter, G. J. E. (1941). *J. Dairy Res.* **12**, 63.
(3) Whitehead, H. R. & Riddet, W. (1933). *N.Z. J. Agric.* **46**, 225.
(4) Whitehead, H. R. (1933). *Biochem. J.* **27**, 1793.


(*MS. received for publication* 23 *August* 1942)

637.333.1:576.8

# 301. THE EFFECT OF OVER-RIPENING UPON THE ACTIVITY OF CHEDDAR CHEESE STARTERS*

By C. K. JOHNS and H. L. BÉRARD, *Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Canada*

(With 3 Figures)

The belief that over-ripening of starters is responsible for their weakening and for slow acid development during Cheddar cheese-making has been widely held by cheese-makers for many years. Statements that over-ripening is harmful have been made by investigators in several countries(1,2,3,4), but experimental evidence in support of this view is scanty. On the other hand, Whitehead(5) in New Zealand has shown that prolonged over-ripening of *single-strain* starters under rather extreme conditions merely resulted in a temporary checking of their activity; between wide limits of size of inoculum and period of incubation no permanent damage was done.

In Canada, mixed culture starters are in general use. Such starters, containing strains varying in ability to produce and tolerate acid, may react to over-ripening somewhat differently to single-strain starters. The studies here reported were undertaken to throw some light on this point.

## I. Effect of over-ripening upon the total acidity produced by starters

The over-ripening of a starter may be brought about by increasing the amount of inoculum, by lengthening the period of incubation, or by increasing the temperature of incubation. The effect of these factors has been studied as follows:

Five different starters were used and all experiments carried out with 100 ml. quantities of skim milk sterilized in Pyrex dilution bottles at 10 lb. pressure for 20 min. Three bottles of milk were inoculated from each starter and incubated at 70° F. Bottle A, the control, received 1 % inoculum and was incubated for 16½ hr. Bottle B received 4 % inoculum and was also incubated for 16½ hr. Bottle C received the same amount of inoculum as A but was incubated for 24 hr. After incubation as indicated, bottles A and B were placed in a refrigerator at 40° F.

Twenty-four hours after inoculation, fresh lots of skim milk were seeded from each bottle. Nine ml. portions of the old culture were withdrawn, the pipette rinsed with an equal quantity of distilled water, and the pipetted portion plus rinsing titrated for acidity. The same procedure was repeated daily for 28 days. However, in order to obtain greater differences in the total acidity produced by the three treatments, the amount of inoculum in A and C was reduced to 0·1 % on the 5th day and to 0·06 % on the 7th day.

After 18 days' incubation at 70° F. there was still no sign of any falling off in activity of the over-ripened portions B and C, even though acidity values as high as 0·97 % were recorded. The temperature was therefore raised to 80° F. for the remainder of the studies in order to make the test more severe.

* Contribution No. 163 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Canada.

The data in Table 1 indicate that over-ripening, continued daily for 4 weeks, had not brought about any decrease in the total acid produced by these starters. Furthermore, incubation at 80° F. after the 18th day failed to show any adverse effect upon the over-ripened portions (B and C). On the contrary, these showed further increases in final acidity of approximately the same order as those shown by the control portions (A). The greater increase in final acidity for the B and C portions of no. 43 than for similar portions of no. 32 is of particular interest, since previous vitality tests by the method of Whitehead & Cox(6) had shown the former to be a much more active starter. Between the 17th and 18th transfers the starters were incubated at room temperature, which fell to below 60° F. during the night. All three portions of no. 32 were much more affected by the low temperature than were the corresponding portions of no. 43. Apparently the latter can stand both high and low incubation temperatures better than no. 32.

Table 1. *Average values for final acidity of five starters*

| | A | | | | B | | | | C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Period* ... | 1st | 2nd | 3rd | 4th | 1st | 2nd | 3rd | 4th | 1st | 2nd | 3rd | 4th |
| Starter no. | | | | | | | | | | | | |
| 21 | 0·74 | 0·74 | 0·86 | 0·86 | 0·89 | 0·91 | 0·95 | 0·96 | 0·85 | 0·88 | 0·88 | 0·91 |
| 32 | 0·71 | 0·75 | 0·81 | 0·78 | 0·86 | 0·93 | 0·98 | 0·97 | 0·88 | 0·94 | 0·95 | 0·95 |
| 38 | 0·75 | 0·75 | 0·79 | 0·86 | 0·85 | 0·87 | 0·97 | 0·97 | 0·83 | 0·89 | 0·94 | 0·94 |
| 43 | 0·75 | 0·75 | 0·80 | 0·81 | 0·87 | 0·85 | 0·94 | 0·98 | 0·85 | 0·88 | 0·96 | 0·96 |
| 49 | 0·72 | 0·77 | 0·77 | 0·77 | 0·85 | 0·86 | 0·86 | 0·91 | 0·81 | 0·86 | 0·85 | 0·83 |
| Average of the 5 starters | 0·73 | 0·75 | 0·81 | 0·82 | 0·86 | 0·88 | 0·94 | 0·96 | 0·84 | 0·89 | 0·92 | 0·92 |

* The temperature of incubation was 70° F. during the 1st and 2nd periods and 80° F. during the 3rd and 4th periods. The 1st period covered 6 days, while each of the remaining periods covered 5 days. The results from the preliminary studies, when inocula of 1 and 0·1 % were used, have been omitted from this table.

## II. Effect of over-ripening upon rates of bacterial growth and of acid development in starters

In Cheddar cheese-making the rate of acid development in the vat is even more important than the final acidity reached in the starter itself. Even slow starters may produce just as much acid during 16 or 24 hr. as very active ones, as reported recently by Harrison & Wolf(7). To throw light upon this point the following experiment was conducted:

After 2 weeks of the over-ripening procedure a preliminary determination of the rate of acid production of these five starters was made according to the following modification of Whitehead & Cox's vitality test(6): Fresh sterile skim milk samples were inoculated with 1 % of 16 hr. old portions A and B of each starter. The freshly inoculated samples were thoroughly shaken and titrated immediately for acidity. In order to simulate the conditions of actual cheese-making, the samples were incubated in a water-bath at 86° F. for 2 hr. and then transferred to an incubator at 102° F. for the next 4 hr. The samples, when transferred from the water-bath to the incubator, took over 1½ hr. to reach 102° F. The samples were carefully mixed before sampling and were titrated for acidity every hour.

The results failed to show any adverse effect of 2 weeks' over-ripening upon the rate of acid production by these starters during the first 6 hr. In fact, the final acidity in the A and B portions was the same for three of the starters, while the B portion of the other two developed more acid than the A portion.

The fact that the rate and amount of acid production were not decreased by over-ripening does not necessarily prove, however, that the activity of the starter organisms

was not weakened. The over-ripened portions might have contained a larger number of organisms than the normally ripened ones and this might have more than compensated for their decreased activity. It was therefore considered desirable to determine the number of organisms in each portion of starter and to follow the rates of growth and of acid production concurrently during incubation.

To obtain data on the relation between acid production and bacterial numbers near the point of coagulation, it was decided to make the study from the 10th to the 16th hour of ripening. Starter 43, portions of which had been over-ripened daily for 21 days, was selected for the experiment.

At the start of the experiment the portions A, B and C of starter 43 were 30½ hr. old. 43A and 43B had been held in the refrigerator for 14 hr. after the incubation period and 43C for 6½ hr. Each portion was transferred in duplicate into 100 ml. portions of sterile skim milk as follows:

A1, B1 and C1 received 1 drop of lots A, B and C respectively.

A2, B2 and C2 received 1 ml. of lots A, B and C respectively.

Smears for direct microscopic counts(8) were made at once from the freshly inoculated samples and the latter incubated at 70° F. Starting at the 10th hour of incubation, titrations and counts of individual cells were made at hourly intervals up to the 16th hour. Agitation of bottles before sampling was done gently in order to minimize the incorporation of air. In preparing the smears, portions of the starters were diluted in sterile skim milk in order to avoid overcrowded fields. The results are presented in Fig. 1.

It is interesting to note that in the three portions which had received a 1 % inoculation, the number of organisms had reached a maximum by the 10th hour and remained relatively constant for the next 6 hr. The maximum acidity, however, was not attained before the 13th hour after inoculation. In the portions which received only 0·06 % of inoculum, it took 13 hr. for the maximum number of organisms to be reached, and only in the B portion had the acidity at the 16th hour reached the level attained by the more heavily inoculated portions.

With both small and large inocula the C portions at both start and finish contained a slightly higher number of organisms than the A and B portions although they produced slightly less acid. Apparently the C portions required a larger number of cells to produce the same amount of acid as the A and B portions. It would seem therefore that with this starter, over-ripening through prolonged incubation had a tendency to decrease the activity of the organisms.

There was practically no difference between the numbers of organisms in the A and B portions. Nevertheless, with the small inoculum the B portion produced slightly more acid, while with the large inoculum the reverse held true. The portions which received the larger inoculum were already coagulated at the 10th hour, while coagulation appeared at the 13th hour in the portions which received a light inoculum.

These results tend to confirm the view expressed by Knudsen(1) that the number of organisms in a starter has reached its maximum at coagulation time. (We are unable to explain why acid production by this starter ceased at a level much below that usually encountered (see Table 1). Subsequent tests have shown that it was not due to the hourly mixing before sampling.) However, since in cheese-making the activity of the starter during the first few hours is particularly important, the experiment was repeated over the first 6 hr., using starters 32, 43 and 49. Fresh skim milk samples were inoculated with

1 % of the 16 hr. old portions A, B and C of each starter. At this time the starters had been over-ripened daily for over 3 weeks, the last 8 days at the temperature of 80° F.

A smear for direct microscopic count was prepared from each sample immediately after inoculation and the latter incubated in a water-bath at 86° F. for 2 hr. The temperature of the bath was then gradually increased to 102° F. within the next hour. It rose to 106° F., however, during the 6th hour because of the failure of the thermostat to open.

At each hour the samples were well mixed and the acid titration and smears made. After the second hour of incubation a 1:11 dilution with sterile skim milk was used for preparing

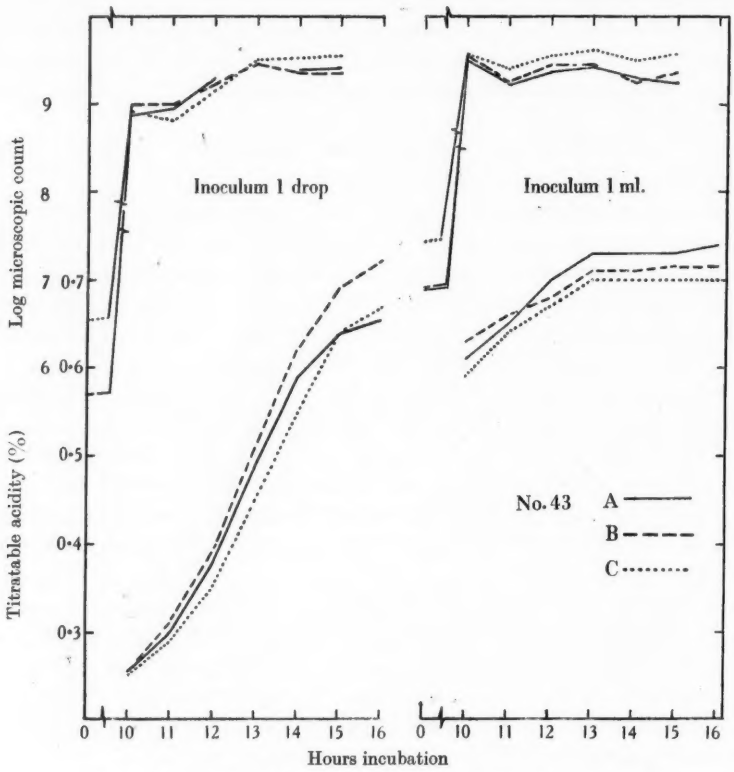


Fig. 1. Rates of bacterial growth and acid production by over-ripened and control portions of starter no. 43.

the smear, and at the 6th hour a 1:121 dilution was used. The results are reported in Table 2 and Fig. 2.

As in the previous experiments, over-ripening appeared to have no effect on the development of acid during the first 6 hr. incubation. The amount of acid produced in the B and C portions was invariably slightly greater than in the A portions. This difference cannot be attributed to a larger number of organisms introduced by inoculation, since the numbers at the start were comparable in all three portions. Furthermore, the rate of bacterial growth was generally greater in the B and C portions than in the A portions. The differences in acid-producing abilities between the three starters are evident from Fig. 2.

Table 2. *Rates of bacterial growth and acid development in portions of three starters*

| | No. 32 | | | | | |
|---|---|---|---|---|---|---|
| | A | | B | | C | |
| Acidity of inoculum ... | 0·76 | | 0·94 | | 0·88 | |
| | Count* | % acid | Count | % acid | Count | % acid |
| 0 hr. | 21·8 | — | 21·0 | — | 21·2 | — |
| 1 „ | 51·9 | 0·16 | 57·3 | 0·16 | 58·8 | 0·16 |
| 2 „ | 220·0 | 0·18 | 229·0 | 0·18 | 210·0 | 0·18 |
| 3 „ | 326·0 | 0·20 | 425·0 | 0·205 | 382·0 | 0·21 |
| 4 „ | 383·0 | 0·24 | 480·0 | 0·25 | — | 0·27 |
| 5 „ | 537·0 | 0·30 | 766·0 | 0·33 | 978·0 | 0·325 |
| 6 „ | 622·0 | 0·355 | 1246·0 | 0·41 | 1278·0 | 0·41 |

| | No. 43 | | | | | |
|---|---|---|---|---|---|---|
| | A | | B | | C | |
| Acidity of inoculum ... | 0·79 | | 0·97 | | 0·89 | |
| | Count | % acid | Count | % acid | Count | % acid |
| 0 hr. | 26·4 | — | 25·9 | — | 28·8 | — |
| 1 „ | 74·0 | 0·16 | 59·9 | 0·165 | 67·5 | 0·165 |
| 2 „ | 215·0 | 0·175 | 218·0 | 0·185 | 270·0 | 0·175 |
| 3 „ | 406·0 | 0·20 | 591·0 | 0·22 | 474·0 | 0·215 |
| 4 „ | 561·0 | 0·25 | 740·0 | 0·28 | 607·0 | 0·26 |
| 5 „ | 644·0 | 0·31 | 1126·0 | 0·38 | 732·0 | 0·335 |
| 6 „ | 958·0 | 0·38 | 1224·0 | 0·48 | 937·0 | 0·405 |

| | No. 49 | | | | | |
|---|---|---|---|---|---|---|
| | A | | B | | C | |
| Acidity of inoculum ... | 0·76 | | 0·86 | | 0·70 | |
| | Count | % acid | Count | % acid | Count | % acid |
| 0 hr. | 24·4 | — | 20·1 | — | 16·2 | — |
| 1 „ | 65·3 | 0·16 | 43·3 | 0·165 | 46·0 | 0·16 |
| 2 „ | 221·0 | 0·175 | 154·0 | 0·175 | 222·0 | 0·17 |
| 3 „ | 375·0 | 0·20 | 385·0 | 0·21 | 277·0 | 0·205 |
| 4 „ | 487·0 | 0·23 | 495·0 | 0·25 | 355·0 | 0·24 |
| 5 „ | 486·0 | 0·26 | 560·0 | 0·30 | 454·0 | 0·285 |
| 6 „ | 655·0 | 0·30 | 995·0 | 0·35 | 689·0 | 0·34 |

* Direct microscopic count of individual cells expressed in millions.

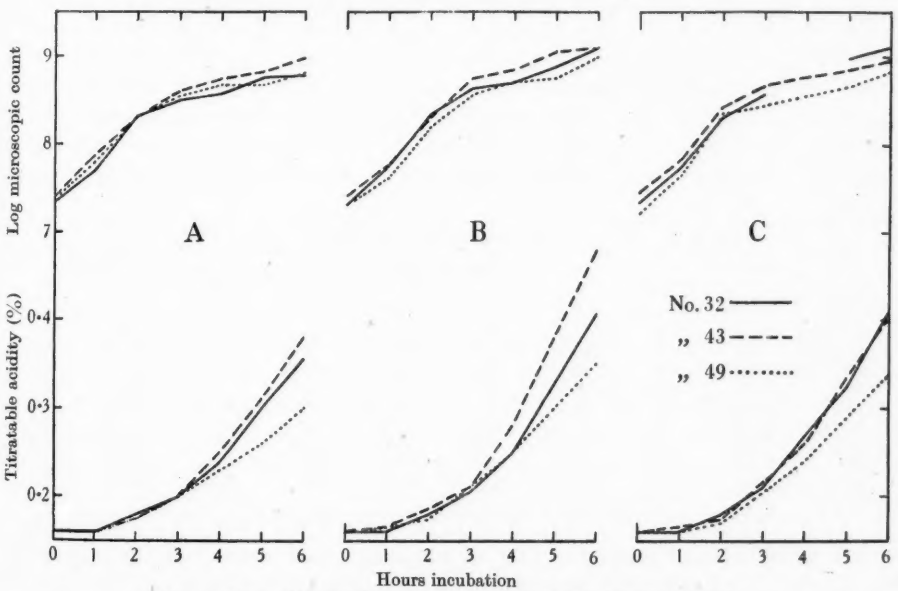


Fig. 2. Rates of bacterial growth and acid production by over-ripened and control portions of starters nos. 32, 43 and 49.

### III. Influence of over-ripening the starter upon actual cheese-making

As a further check upon the reliability of the findings reported above, two vats of cheese were made from portions of the same lot of milk on the 19th day of these studies, using starter 43. Vat A received 1 % of 16 hr. old starter 43A, the acidity of which was 0·75 %. Vat B received the same amount from the B portion which showed an acidity of 0·88 %.

Vat B came along more rapidly than vat A and was piled 27 min. earlier. This difference was not due to a larger number of organisms added to the vat, as a count made immediately after adding the starters showed that the milk in vat A contained 36,000,000 organisms per ml. while that in vat B contained only 29,000,000 per ml.

After 3 weeks' curing, the cheese from vat B was slightly superior in flavour to that from vat A; after 7 months there was over a point difference in flavour score in favour of vat B. These results confirm those from the acid determinations reported in Table 2, wherein the B portion also developed acid more rapidly than the A portion.

### IV. Influence of over-ripening the starter upon the proportion of rapid acid-producing organisms

Sherman & Cameron (9) have shown that rapid acid-producing strains in starters are less acid-tolerant than the slow acid-producing strains. Continued over-ripening might thus be expected to increase the proportion of slow acid-producing organisms. Our experiments have shown that the over-ripened portions of starters almost invariably produced acid more rapidly than did the control portions. That this was not due to the presence of significantly larger numbers of organisms in the over-ripened portions is evident from the data in Table 2 and Fig. 2. As a further step, the percentages of rapid acid-producing bacteria were determined about a month after the over-ripening studies were commenced. After incubation at 80° F. for 17 hr., the various portions of starters 32, 43 and 49 were plated out on tryptone glucose whey agar containing 0·25 % of yeast extract and the plates incubated for 3 days at around 86° F. Approximately 100 colonies from one sector of a plate from each portion of each starter were fished into tubes of litmus milk. These tubes were incubated at 37° C. (98·6° F.) for 2 days and at 30° C. (86° F.) for 5 days. The number of coagulated tubes was recorded every 24 hr. At the end of the incubation period all tubes showing no visual change were checked microscopically for the presence of organisms. The plate counts and the litmus milk reactions are reported in Table 3 and Fig. 3.

Table 3. *Effect of over-ripening upon flora of cheese starters*

| Starter | Plate count in millions | No. of colonies fished* | Percentages of litmus milk tubes showing coagulation after | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
| 32A | 1,050 | 81 | 12·3 | 23·4 | 35·8 | 59·2 | 71·6 | 81·4 | 83·9 |
| 32B | 400 | 75 | 54·6 | 54·6 | 54·6 | 56·0 | 56·0 | 56·0 | 56·0 |
| 32C | 1,580 | 101 | 24·7 | 71·2 | 87·1 | 90·0 | 90·0 | 93·0 | 93·0 |
| 43A | 1,800 | 100 | 24·0 | 90·0 | 94·0 | 99·0 | 99·0 | 99·0 | 100·0 |
| 43B | 1,510 | 97 | 13·4 | 72·1 | 76·3 | 80·4 | 82·4 | 82·4 | 82·4 |
| 43C | 2,160 | 104 | 11·5 | 50·0 | 50·9 | 51·9 | 53·8 | 53·8 | 53·8 |
| 49A | 3,380 | 92 | 0·0 | 0·0 | 76·0 | 83·6 | 84·7 | 85·8 | 85·8 |
| 49B | 1,980 | 76 | 27·6 | 27·6 | 30·2 | 42·1 | 50·0 | 55·2 | 55·2 |
| 49C | 850 | 56 | 21·4 | 21·4 | 26·7 | 32·1 | 33·9 | 35·7 | 35·7 |

* Excluding those from which litmus milk tubes showed no growth on microscopical examination.

The data show that with no. 43 the over-ripening had a deleterious effect upon the proportion of rapid acid-producing organisms, more so in the C than in the B portion. It will be recalled that, while discussing the results reported in Fig. 1, it was mentioned that

the C portion, although containing a larger number of organisms, produced a slightly lower amount of acid than the A and B portions. The more pronounced effect of over-ripening upon the B and C portions in the present experiment may be the result of incubation at 80° F. for the last 12 days.

The C portion of no. 32 showed a much larger proportion of rapid acid-producing organisms than the A and also larger than the B portion after the first 24 hr. As previously mentioned, no. 43 proved to be an active starter and no. 32 a slow starter when tested for vitality by Whitehead's technique. Previous studies (unpublished) had shown that prolonged incubation at a fairly high temperature (37° C.) increased the percentage of rapid acid-producing types in a slow starter, although the flavour was adversely affected.

Starter 49 was intermediate between the other two starters. The B portion at first contained the largest percentage of rapid milk-coagulating strains, but after 2 days' incubation the proportion became much larger in the A than in the B or C portions.

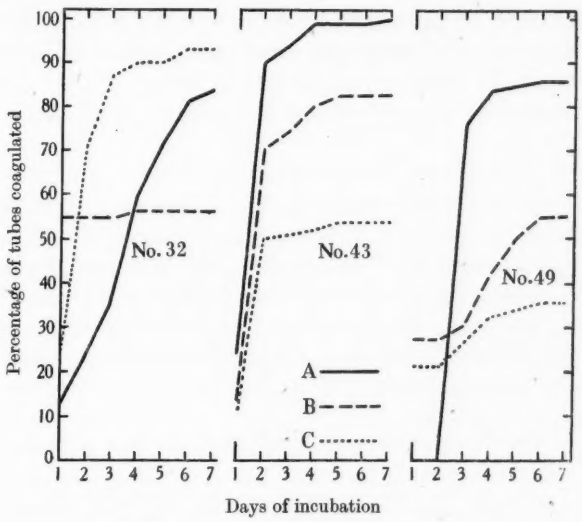


Fig. 3. Effect of over-ripening upon flora of cheese starters.

It is also interesting to note that in the A portions of each starter over 80 % of the strains coagulated the milk during the 7-day period. In the B portions they varied from 55 to 82 % and in the C portions from 35 to 93 %.

The use of higher incubation temperatures (37° C. for 2 days, then 30° C. for 5 days) for the litmus milk tubes may be responsible for at least some of the apparent anomalies in these results. Had the tubes been incubated at 70 or 80° F., the picture might have been quite different, especially during the first 2 days. As it is, only those organisms capable of growth at approximately cooking temperatures would be recorded as coagulating milk by the end of the 2nd day. The effect of lowering the temperature to 86° F. after the 2nd day is particularly noticeable in the case of no. 49A, where the percentage of positive tubes jumped from 0·0 to 76·0 % on the 3rd day.

Over-ripening evidently brings about some changes in the flora of a specific starter, but the differences between similarly treated portions of the three starters are even larger than those between the various portions of the same starter. In view of the marked

variability in the effect of over-ripening upon the percentage of milk-coagulating organisms in these three starters, it is obviously unsafe to generalize concerning changes brought about in the flora.

### V. Influence of over-ripening upon the flavour of starters

Though the belief that the flavour of a starter is a useful guide to its suitability for cheese-making is no longer considered well founded, it is a view widely held by cheese-makers. As a matter of interest, therefore, it was decided to compare the flavours of the A, B and C portions with those of the original starters. (During the period of these studies the original starters had been transferred three times a week, incubated at 70° F. for 16 hr., then held at 40° F. until the next transfer.)

At this time the over-ripening had been carried on for 30 days, the last 12 days at 80° F. Portions of sterile skim milk were inoculated with 1 % of each portion of starters 32, 43 and 49. An additional portion was inoculated from the original starter in each case. The scoring for flavour was done after 15 hr. incubation at 70° F. In the opinion of six judges, the B portion from each starter was considered the best and the A portion the worst in each instance. All three portions of starter 32 had a better flavour than the original, while the B portions of starters 43 and 49 were just as good as, if not better than, the original.

### Discussion

While the data presented show that neither the final acidity nor the rate of acid development, especially during the first 6 hr., is impaired by over-ripening, incubation at 80° F. instead of at 70° F. does retard acid development with some starters. This is illustrated by the data on the relative performances of starters 32, 43 and 49 in the two series of tests (Table 4). The first series of experiments was performed on the 14th day, when incubation was still at 70° F.; the second was carried out on the 27th day, during the last 8 days of which incubation had been at 80° F.

Table 4. *Percentage acidities of starters at the 6th hour of incubation*

| | First series (70° F.) | | Second series (80° F.) | |
|---|---|---|---|---|
| Starter no. | Portion A | Portion B | Portion A | Portion B |
| 32 | 0·40 | 0·47 | 0·355 | 0·41 |
| 43 | 0·38 | 0·42 | 0·38 | 0·48 |
| 49 | 0·40 | 0·40 | 0·30 | 0·35 |

The lower acidities of both A and B portions of starters 32 and 49 when incubated at 80° F. suggest that neither of these would prove to be an active starter in the vat, where temperatures from 85° to over 100° F. would be encountered. This is also indicated by the results of the vitality test. On the other hand, no. 43, which had proved to be an active starter both in the vat and in the vitality test, actually showed an increase in acid production at the higher temperature of incubation.

Since these studies were completed, Rice (10) has reported that 'so long as over-ripening is avoided, a starter will produce acid normally at any period after the logarithmic period (of growth) is well established until maximum acidity is reached and no advantage is derived by cooling a starter as soon as coagulation occurs'. He states further '...continued over-ripening (of a mother starter) is probably a factor causing the gradual weakening of all starters kept for long under factory conditions'. Our results also indicate that no advantage is derived by cooling a starter immediately after coagulation;

they also suggest that over-ripening is unlikely to be responsible for the weakening of starters to which Rice refers.

It is not easy to explain why, during the first 6 hr. incubation, the over-ripened portions often produced acid more rapidly than the controls. Although the number of organisms introduced in the inoculum was of the same order in each portion, the data suggest that the over-ripened portions contained a larger proportion of rapidly growing strains than did the controls. This hypothesis, however, does not appear to be supported by the data in Table 3 and Fig. 3. For example, while the A portion of no. 43 contained a larger percentage of rapid milk-coagulating organisms, it produced acid less rapidly than the B and C portions when inoculated into milk (Figs. 1, 2). This apparent discrepancy may be due to the higher temperatures at which the litmus milk tubes were incubated in the determination of the percentages of rapid acid-producing organisms. Another factor which may have influenced the results is that the rates of acid production and the percentages of rapid milk-coagulating organisms were not determined concurrently. The higher incubation temperature during the 2 weeks immediately preceding the studies on the flora may have caused significant changes in the proportions of rapid milk-coagulating organisms.

## CONCLUSIONS

Severe and prolonged over-ripening of starters to an extent greater than usually encountered in cheese-factory practice failed to slow down the rate of bacterial growth or acid development, or to lower the final acidity reached.

In a practical test an over-ripened portion of a starter worked slightly faster in the vat and produced cheese with a higher flavour score than the control.

The effect of over-ripening upon the proportion of rapid milk-coagulating organisms varied considerably between the three starters studied. In two of these an over-ripened portion contained more of these organisms than the normally ripened portion.

After 30 days of repeated over-ripening, the flavour of the over-ripened portions was judged superior to that of the normally ripened portions.

Grateful acknowledgement is made to Mr C. A. Gibson, Creamery Manager, Central Experimental Farm, who conducted the cheese-making test, and to Mr T. J. Hicks, Senior Dairy Produce Grader, Dairy Products Division, Marketing Service, who scored the cheese.

## REFERENCES

(1) KNUDSEN (1931). *J. Dairy Res.* **2**, 137.
(2) SAMMIS (1924). *Cheesemaking.* 7th ed., Madison, Wisc.: Mendota Book Co.
(3) PRICE, WEBSTER & HURD (1932). *Bull. Oregon agric. Exp. Sta.* no. 301.
(4) RANDELL & SHELTON (1931). *N.S. Wales agric. Gaz.* **42**, 488, 508.
(5) WHITEHEAD (1939). *J. Dairy Res.* **5**, 120.
(6) WHITEHEAD & COX (1932). *N.Z. J. Sci. Tech.* **13**, 304.
(7) HARRISON & WOLF (1941). *Proc. Soc. agric. Bact.* (Abstracts), p. 19.
(8) American Public Health Association (1941). *Standard Methods for the Analysis of Dairy Products*, 8th ed. New York, N.Y.
(9) SHERMAN & CAMERON (1934). *J. Bact.* **27**, 23.
(10) RICE (1942). *Dairy Industr.* **7**, 230.

(*MS. received for publication* 21 *January* 1943)

637.333.1:576.809.6

# 302. BACTERIOPHAGES FOR *STREPTOCOCCUS CREMORIS* PHAGE DEVELOPMENT AT VARIOUS TEMPERATURES

By G. J. E. HUNTER,
*From the Dairy Research Institute (N.Z.), Palmerston North, N.Z.*

(With 6 Figures)

Bacterial growth has long been considered the prime conditioning factor for phage multiplication. Scribner & Krueger (1) were the first to suggest that the two phenomena were not necessarily coincident. Working with *Staphylococcus* phage in the presence of 0·25 *M* NaCl, they observed that during the phase of the phage-bacterium reaction just before lysis, phage multiplied without concomitant bacterial growth. Krueger & Fong (2), by careful adjustment of *p*H and temperature of the medium, showed that bacterial growth could be completely inhibited while phage multiplication continued at a rapid rate. They concluded that bacterial growth was not essential for phage development.

In early studies on the development of bacteriophage in starter cultures, a very close correlation between bacterial growth and phage development was evident, and this correlation, in conjunction with the apparent type specificity of the phages then available, led to the suggestion that phage could originate within the bacterial culture itself (3). Subsequent work (4) showed that some phage races could attack up to four different strains of *Streptococcus cremoris*, and since air-borne bacteriophage in the surroundings of cheese factories accounted for many of the sudden, apparently spontaneous, appearances of phage in starter cultures, the hypothesis of a bacterial origin for phage was abandoned (5). The present communication gives an account of further investigations on the growth of lactic streptococci and their phages in milk. From the data presented it will be seen that, contrary to previous indications, bacterial reproduction and phage multiplication are separate and distinct processes. Certain phage races fail to multiply at a temperature of 37° C., while the rate of phage multiplication of other races is not always closely related to the growth rate of the homologous organism. In many instances the conditions for maximum activity of bacteria and phage are not identical, and the fact that an organism grows most rapidly at a given temperature is not necessarily an indication that a phage race which is able to develop at the expense of that organism will find the conditions most favourable at that particular temperature.

## Growth of lactic streptococci and their phages at various temperatures

*Characteristics of the organisms and phages.* The organisms used in this study were strains of *Str. cremoris* (Orla Jensen), all of which have been in use from time to time as 'single-strain' starter cultures for cheese-making in New Zealand. They were identical biochemically and were distinguishable from one another only by differences in morphology after growth in milk at 37° C. for 6 hr., and by the rate and extent of acid formation in milk. The maximum temperature for growth varied slightly in the region of 38–40° C., and while some organisms could be subcultured in milk at 37° C. for two or more generations, others rapidly died out under this treatment.

The phage races examined were isolated from material in commercial factories and purified (4) several times upon the particular strain of *Str. cremoris* on which they first made their appearance. The maximum titre of phage attainable with any particular organism and phage depends in the first place on the nature and type of substrate organism and phage race, while the experimental conditions which determine whether or not the maximum titre is attained are (*a*) the initial concentration of lytic agent and amount of bacterial inoculum, (*b*) the temperature at which the phage-organism reaction takes place. The rapid acid production which occurs in cultures of *Str. cremoris* results in an early attainment of low *p*H values sufficient to inhibit further growth of the organisms and cause a cessation of phage multiplication. The range is therefore limited when attempts are made to obtain a preparation of maximum strength by complete lysis in one generation of a culture in milk. Too high a concentration of phage does not permit sufficient reproduction before lysis of the organisms, while too low a concentration allows too great an acid formation and hence clotting of the milk before lysis can take place. It was found that intermediate amounts of both bacteria and phage gave final preparations with the highest phage titre. In general a temperature of 30° C. was the most suitable, and an inoculum of 1–2 % of an 18–20 hr. milk culture of the substrate organism with 0·05 % of a $10^{-2}$ to $10^{-6}$ dilution of the average phage preparation gave the best results. With a standard 1 % bacterial inoculum, the final titre obtained (when tested by the plate technique on solid agar) was approximately the same whether the smaller or the larger initial concentration of phage was used; the only difference observed was the longer or shorter time required to reach the 'lytic threshold value' requisite for lysis of the organisms. In the experiments detailed below, average phage preparations with titres of $10^{-10}$ to $10^{-12}$ were employed. They were similar in all their general properties and differed only in the range of streptococci which they attacked. Some races proved to be type specific while others were polyvalent.

*Experimental method.* Acid formation and rate of phage multiplication were followed in 100 ml. quantities of autoclaved skim milk previously warmed to the required temperature and then seeded with 1 % of an 18–20 hr. clotted-milk culture and varying amounts of phage added. At intervals 2 ml. were withdrawn for titration against weak alkali and 1 ml. was used for dilution in the plate test for phage multiplication. Too strong an initial phage concentration frequently did not permit a sharp distinction to be observed in the optimum temperature for development and consequently an initial concentration judged to give complete lysis of the organisms developed in 4–7 hr. from a standard 1 % bacterial inoculum was used in most experiments. All phages were adjusted to approximately equal strength based on the titres obtained on the homologous organisms and 0·05 ml. of a $10^{-6}$ dilution used to 100 ml. milk.

The exact extent to which titratable acidity follows the actual growth curve of the particular streptococci is not known with certainty, but since acid production is the main feature of the metabolism of the organisms it was taken as a growth indicator. Strains of *Str. cremoris* form chains of varying length at different temperatures and accurate bacterial counts were therefore not obtainable. However, microscopical examination at intervals gave ample evidence that growth rate and acid formation were closely related. Small amounts of phage exhibited very little, if any, stimulation of growth or acid production, and hence the growth curves obtained from the control flasks and from the flasks to which phage was added, were identical up to the point of lysis. The various

experiments were repeated several times over a period of 6 months, and except for slight differences in the rate of acid production in milks of different chemical composition, the general shape of the curves and times of lysis were approximately the same throughout.

*Results.* The results of titration, calculated as percentage lactic acid, were plotted against the length of time from inoculation and representative curves are shown in Figs. 1–6. The unbroken lines show the acidity produced in both the control and phage flasks up to the point of lysis which is indicated by an arrow. With the complete dissolution of the

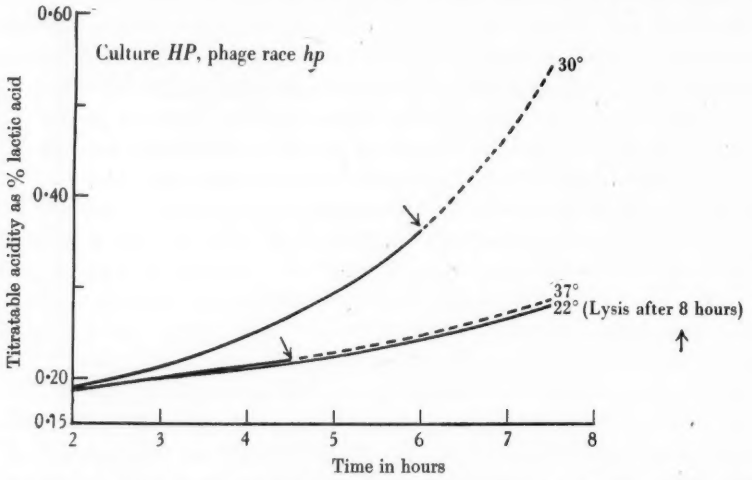


Fig. 1. Culture *HP*, phage race *hp*. Although the growth rate was practically the same at 22 and 37° C., phage race *hp* developed much more rapidly at the higher temperature and attained the concentration required for lysis in less than 4½ hr. At 22° C. it developed slowly and did not reach the 'lytic threshold concentration' until many hours later. The optimum growth temperature for culture *HP* was 30° C., at which temperature the phage grew rapidly and lysis occurred in approximately 6 hr. Phage *hp* multiplied equally well at 30 and 37° C., and this, in conjunction with the retardation of bacterial reproduction, allowed lysis to take place more rapidly at 37° C.

organisms in the flasks containing phage no further acid production occurred, but the course of acid formation in the controls is indicated by the dotted lines. The rate of phage multiplication was followed at suitable intervals by preparing a series of dilutions in sterile water and placing a loopful of each on a mat of the susceptible organism previously spread on a solid agar medium. Frequent microscopical examinations were also carried out to ascertain when complete lysis occurred.

The results show the differences between (*a*) the various strains of *Str. cremoris*, (*b*) the various phage races, and (*c*) each organism and its appropriate phage.

It is evident from Figs. 1 and 2 that although the growth of strains *HP* and $R_1$ was most rapid at 30° C., the temperature favouring phage multiplication and consequent lysis was markedly different. A slightly increased rate of multiplication of *hp* was observed at 37° C., while race $r_1$ was inhibited or retarded at high temperature. This effect is illustrated in Fig. 6, which shows a comparison of the growth of race *hp* on culture *HP* and $r_1$ on $R_1$ in milk held at 30° C. and in milk held at 30° C. for 1 hr., then 37° C. for 2 hr. and finally transferred to 30° C. for the remainder of the experiment. These temperatures were employed because they correspond to some extent with those used during the cheese-

manufacturing process. Culture *HP* 'phaged' in less than 6 hr. at 30° C. Bacterial growth was retarded at 37° C., but the ready development of phage *hp* shortened the time of lysis as indicated in the graph by a shift of the arrow to the left. Exposure to the higher temperature inhibited multiplication of $r_1$ phage; subsequent holding at 30° C. permitted development, and lysis occurred very much later than in milk held at 30° C. throughout. This is indicated by a shift of the arrow to the right.

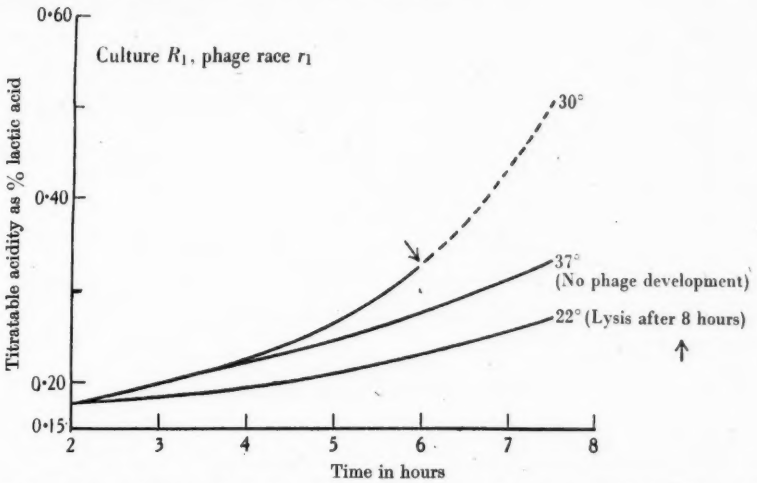


Fig. 2. Culture $R_1$, phage race $r_1$. A good growth of organisms and of phage occurred at 30° C. and dissolution of the organisms took place in about 6 hr. At 22° C. there was a steady development of both, but lysis required a longer time. A relatively rapid growth of culture $R_1$ was obtained at 37° C., but no phage development could be detected at this temperature. Some activity of the particular race $r_1$ was evident, however, if high initial concentrations were added to the milk.

Holding milk containing $r_1$ phage and inoculated with $R_1$ culture at 37° C. for 2 or 4 hr. caused inhibition of $r_1$, but multiplication occurring on subsequent transfer to 30° C. gave good phage titres before the milk clotted. Further experiments demonstrated that the initial phage added to milk held at 37° C. for 24 hr. or more was not destroyed, and hence it appeared that the effect was one of true inhibition.

## RANGE OF ACTIVITY OF PHAGES

Of the phages tested, races *hp* and $r_1$ were distinct in that they have so far proved to be type specific. Thus strains *HP* and $R_1$ could readily be distinguished from other strains of *Str. cremoris* by the action of these phages upon them. The organisms *RW*, $R_8$ and $R_6$ were, however, very closely related, since they were sensitive to one or more of a group of phages. On solid media at 30° C. all three were attacked by races *rw*, $r_8$ and $r_6$. The range of activity was also tested in milk at 22, 30, and 37° C., using 100 ml. quantities of autoclaved skim milk seeded with 1 % bacterial culture and 0·05 ml. of a $10^{-6}$ dilution of phage preparation. The results are tabulated in Table 1. A plus sign indicates that phage

Table 1. *Range of activity of phages rw, $r_8$, $r_6$, in milk at* 22, 30 *and* 37° C.

| | Phage races | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *rw* | | | $r_8$ | | | $r_6$ | | |
| Organisms | 22° | 30° | 37° | 22° | 30° | 37° | 22° | 30° | 37° |
| *RW* | + | + | − | + | + | + | + | + | − |
| $R_8$ | + | + | − | + | + | + | + | + | − |
| $R_6$ | − | − | − | + | + | + | + | + | − |

development occurred and lysis took place within 7 hr. A negative sign signifies that no phage multiplication took place.

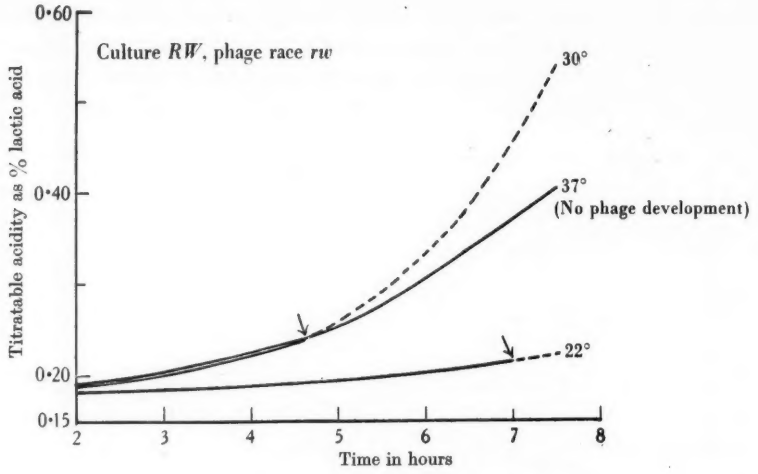


Fig. 3. Culture *RW*, phage race *rw*.

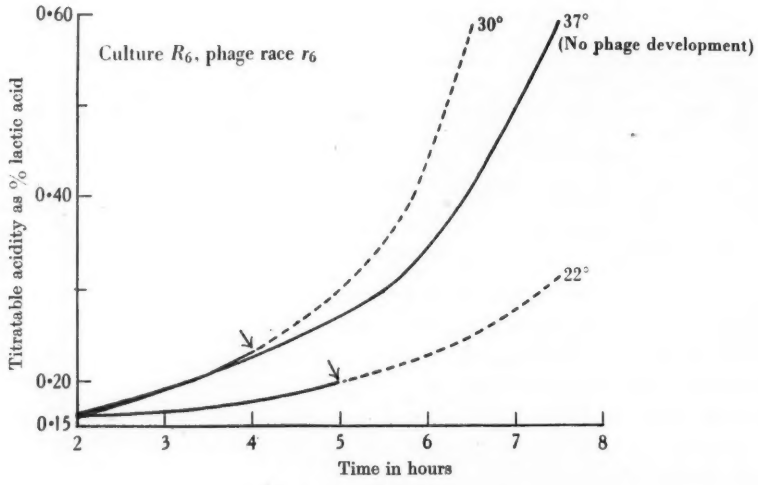


Fig. 4. Culture $R_6$, phage race $r_6$.

Phage races *rw* and $r_6$ did not develop at 37° C. Strains *RW* and $R_6$ have a relatively high maximum growth temperature and can be subcultured at 37° C., yet the phages, in contrast to phage $r_1$ on strain $R_1$, did not develop at this temperature even when strong initial concentrations of undiluted phage were used. It is noteworthy that these phage races developed more rapidly at 22° C. than races *hp* or $r_1$.

The lack of development of races *rw* and $r_6$ at 37° C. on any of the organisms was outstanding. There was no effect on acid production, while the plate technique and dilution method in milk failed to detect any phage multiplication, although it was readily proved that the initial phage added was not destroyed at 37° C. In all cases where no lysis or

phage development occurred, the tests were repeated using higher initial concentrations of phage but no differences were observed.

Cross resistance tests indicated that races *rw*, $r_8$ and $r_6$ were all of one type.

The susceptibility of strain $R_6$ to lysis on agar by phage *rw* was well marked with the stronger concentration of phage (e.g. $10^{-1}$, $10^{-2}$ dilutions) but plaque formation with the

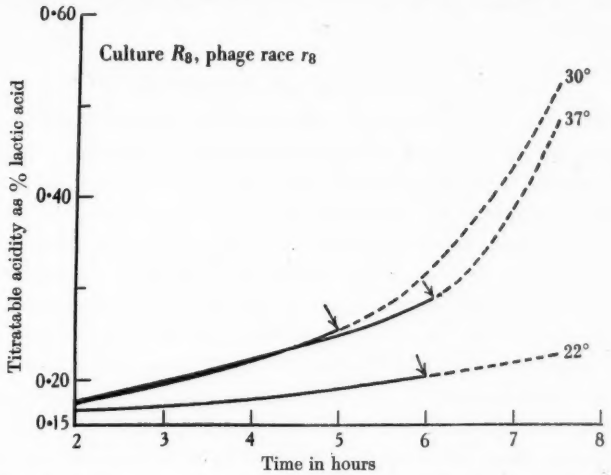


Fig. 5. Culture $R_8$, phage race $r_8$. Race $r_8$ developed rapidly at all temperatures but preferred 30° C.

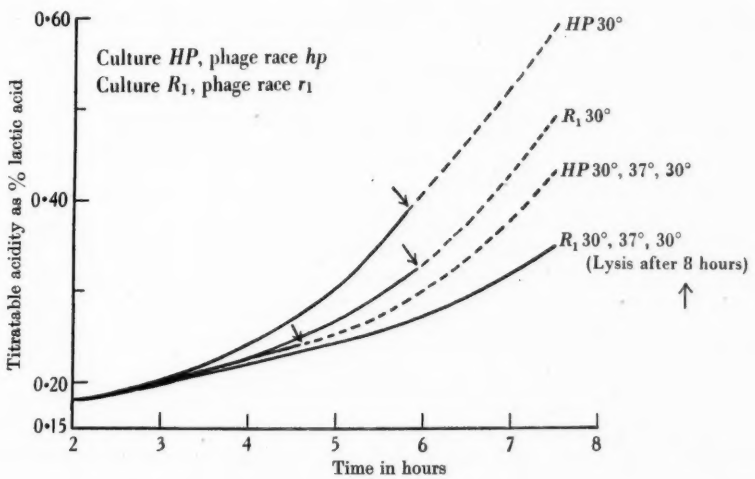


Fig. 6.

weaker concentrations was either absent or not clear cut. The organisms proved to be resistant to the phage when grown in milk at the three temperatures. However, in the presence of the sensitive non-lysogenic strain *RW*, phage race *rw* was able to attack and bring about complete lysis of $R_6$ organisms growing in the liquid medium at 30° but not at 37° C. This was demonstrated by a technique similar to that described above except that a

trace of culture *RW* was added to the milk with the usual 1 % inoculum of strain $R_6$ and varying amounts down to 0·05 ml. of a $10^{-6}$ dilution of *rw* phage. The same effect could be obtained with strain $R_8$ as the sensitive organism. Wollman & Wollman(6), in an investigation using staphylococcus phage, showed that a phage may lyse resistant living strains in the presence of a growing sensitive strain, and a similar increased activity was noted and utilised by Evans(7) working with a group of phages active against strains of haemolytic streptococci.

### Constancy in character of phage races

The observations recorded above made it apparent that as each of the organisms *RW*, $R_8$ and $R_6$ were susceptible to at least three phages, a culture such as $R_8$, on being put into use as a starter culture in a cheese factory, could be attacked by a phage such as *rw* or $r_6$ which does not develop readily at higher temperatures, or it may be lysed by a race, $r_8$ for example, which can multiply readily at any temperature. Various phage-organism combinations were prepared from stock phages and organisms and the resulting lytic filtrates tested against the organisms on plates at 30° C. and in milk at 30 and 37° C. The lytic filtrates are indicated as follows: *rw*/*RW* is the filtrate prepared by the action of race *rw* on culture *RW*. Since *rw* did not develop on $R_6$ in milk the filtrate *rw*/$R_6$ was not obtained. On plates at 30° C. all the preparations gave clear areas of lysis on *RW*, $R_8$ and $R_6$. The results in milk at 30 and 37° C. are shown in Table 2. A plus sign indicates that the phage preparations acted readily on the organisms growing in milk. A negative sign indicates that no action or phage development occurred even with high initial phage concentrations.

Table 2. *Action of lytic filtrates on organisms growing in milk at* 30 *and* 37° C.

| | Lytic filtrates | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *rw*/*RW* | | $r_8$/*RW* | | $r_6$/*RW* | | *rw*/$R_8$ | | $r_8$/$R_8$ | | $r_6$/$R_8$ | | $r_8$/$R_6$ | | $r_6$/$R_6$ | |
| Organisms | 30° | 37° | 30° | 37° | 30° | 37° | 30° | 37° | 30° | 37° | 30° | 37° | 30° | 37° | 30° | 37° |
| *RW* | + | − | + | + | + | − | + | − | + | + | + | − | + | + | + | − |
| $R_8$ | + | − | + | + | + | − | + | − | + | + | + | − | + | + | + | − |
| $R_6$ | − | − | + | + | + | − | − | − | + | + | + | − | + | + | + | − |

A constancy in character of phages is illustrated by the finding that races *rw* and $r_6$ did not develop in milk held at 37° C. no matter on what substrate they were prepared. The temperature at which a phage was isolated and purified appeared to have little influence, since a race such as $r_8$/$R_6$ prepared at 22° C. acted quite readily at 37° C. and vice versa.

### Implication of the results in cheese-making practice

The findings described above give a basis of explanation for some of the phenomena encountered in commercial cheese factories. Ignoring for the moment the starter mother cultures, the starter streptococci are required to grow under two quite different sets of conditions during the process of cheese manufacture:

(*a*) In sterilized milk in the bulk starter cans at 22° C.

(*b*) In milk and curd in the cheese vats at 30° C. for some periods and at 37° C. for a period of about 2 hr.

Where there is a phage infection in the can or the vats, the result obviously depends to some extent on how the particular phage race involved reacts to temperature changes.

(*a*) To deal first with the bulk starter culture incubated daily at 22° C.; an accidental infection of a culture of $R_6$ with phage $r_6$ is much more likely to lead to lysis (a 'thin' bulk starter) than an equivalent infection of culture *HP* with phage *hp*. With a very small infection in the case of *HP* the development of phage in the starter can at 22° C. may be so slight that the starter will behave normally next day in the cheese vat, the contaminant phage lysing the organism only after the cheese-making process is complete. This difference in result rests solely on the different growth rates of phages $r_6$ and *hp* at 22° C. This difference is evident in actual practice, and factory managers say that culture $R_6$ is 'sensitive' and difficult to keep compared with *HP*. (*b*) Assuming now that the bulk starter is not infected with phage, in normal commercial practice it inevitably becomes infected in the cheese vat since the whole factory is infected from the working of previous days. Only rarely does this infection cause any trouble because it appears in most cases to be so small that even if the phage does grow readily at 37° C. it does not reach a concentration sufficient to cause lysis before the cheese-making operations are finished. Yet there is a considerable development of phage in the whey in the vat, and the amount depends to some extent on whether the particular phage race grows readily or is inhibited at 37° C. In border line cases, where phage infection in the vat approaches the danger point the rate of growth of phage at 37° C. may determine whether or not a 'failure' of starter occurs during the cheese-making process. Instances have been encountered which could be explained on these lines. Obviously on the results described in the present paper culture $R_1$ will withstand a heavier infection at 37° C. with its appropriate phage than will culture *HP* with its phage.

Furthermore, the extent of phage development in the vat will react back and influence the chance of contamination of the bulk starter, because the whey drawn from the vat and atomized during passage through the whey separator is the source of air-borne phage. A phage race which does not develop readily at 37° C. will be at a lower concentration in its air-borne form, although the advantage to be derived from this circumstance may be negatived if the particular phage grows more readily than others at 22° C. in the starter cans. It will be sufficiently evident from the foregoing that the possibilities in practice are complex; yet many phenomena encountered in the past in cheese factories fit into the picture when considered in the light of the present findings.

The fact that phage races have different optimum growth temperatures quite distinct from the optimum growth temperatures of the substrate organisms has a bearing also on the tests which are used to detect air-borne phage. In our work sterilized milk exposed to the atmosphere in Petri dishes and subsequently inoculated with the test organism have usually been incubated at 22° C. It is now evident that if the test is to be equally sensitive for all phage races, several samples exposed and inoculated in similar fashion should be incubated at a series of temperatures between 22 and 37° C. in order that the phages possibly present may have an equal chance of lysing the streptococci.

The finding that a phage may lyse an organism only in the presence of another 'sensitive' organism complicates matters considerably in the practical sphere. It has become the practice in recent years in New Zealand to use two or three separate and distinct strains of *Str. cremoris* as starters, to keep them as separate mother cultures and bulk starters and to mix them in the milk in the cheese vat. This system is used to give a measure of safety, since even under the best conditions it is impossible always to be sure that the bulk starter is not infected with phage, even when it appears to be quite normal. In

general the system is quite successful, but it now appears that there may be cases where, with two strains of *Str. cremoris* in the cheese vat and phage which normally attacks only one of them, the other strain may be lysed because it is present in the mixture. This may account for some of the curious and sudden occurrences of 'sensitiveness' which has been observed with commercial starters consisting of a mixture of several strains of *Str. cremoris*. The infection of such a starter with a phage or with another strain of *Str. cremoris* may obviously have a variety of results.

## Discussion

The experimental evidence brought forward in this paper does not shed any light on the ultimate origin of bacteriophage, but it suggests that the phage races as they exist are separate and distinct entities whose growth conditions are similar to, but not necessarily identical with, those of the bacteria upon which they develop. The fact that phage race and homologous organism react differently to a change in incubation temperature meant that the picture is very complicated. In general the streptococci grow more quickly at 30 than at 22° C., but are inhibited to a greater or less degree at 37° C. The phage races exhibit a somewhat wider diversity of reaction to temperature; they tend to develop more readily at 30 than at 22° C., although in some cases the difference is negligible; some develop just as readily at 37 as at 30° C., while others are completely inhibited at 37° C. The very complication of the picture lends emphasis to the view that organism and phage are independent entities in so far as their temperature preferences are concerned.

It is opportune at this time to discuss the question of nomenclature of phage races. In work at this Institute it has been the practice to name a phage race according to the substrate organism on which it was first isolated. This seemed logical because of the apparent specificity of the phages, but with the isolation of phages which attack more than one strain of *Streptococcus* and of streptococci attacked by more than one phage this system of nomenclature gives a false idea of specificity. For instance, in the experiments described in the present paper, the naming of phages *rw*, $r_8$ and $r_6$ is arbitrary. It so happens that race *rw* was isolated in connexion with culture *RW*. But if *RW* is used as a starter in a commercial factory under conditions in which contamination may occur, it is a matter of chance whether the contaminant phage which eventually makes its appearance is *rw*, $r_8$, $r_6$, another race altogether, or a mixture of two or more races. Thus, in the light of present knowledge, it will be more reasonable in the future to give phage races labels which do not imply a special association with any one organism even though in certain cases (e.g. race $r_1$ and organism $R_1$) there appears up to the present to be a strict specificity. Such a specificity may at any time be disproved by isolation of another strain of *Streptococcus* or another phage race.

The difficulty of differentiating phage races mentioned previously (4) still obtains, but the criterion of reaction to temperature of incubation is a new factor which can be taken into consideration. The three factors available to characterize streptococcal phage races in order of their usefulness are:

(*a*) Range of streptococcal strains on which the phage acts.

(*b*) Reaction of the phage to changes in incubation temperature.

(*c*) Thermal death-point of the phage.

The last criterion only rarely helps, because most of the streptococcal phage races are destroyed within a very narrow range of temperature. The second criterion, discovered

as a result of the work described in this paper, is far more useful and promises to be of value in completing the complicated picture of the relationship between the lactic streptococci and the phages which attack them.

SUMMARY

The effect of temperature on the growth in milk of several strains of *Str. cremoris* and their appropriate phages has been investigated. The phage races show a wider diversity of reaction to temperature conditions than do the homologous organisms. They frequently have different optimum growth temperatures quite distinct from the optimum growth temperature of the substrate organisms. Some races fail to multiply at 37° C.

The implication of the results in cheese-making practice is discussed.

The author is indebted to Dr H. R. Whitehead for helpful criticism and advice.

REFERENCES

(1) SCRIBNER & KRUEGER (1937). *J. Gen. Physiol.* **21**, 1.
(2) KRUEGER & FONG (1937). *J. Gen. Physiol.* **21**, 137.
(3) WHITEHEAD & HUNTER (1937). *J. Path. Bact.* **44**, 337.
(4) WHITEHEAD & HUNTER (1939). *J. Dairy Res.* **10**, 403.
(5) WHITEHEAD & HUNTER (1941). *J. Dairy Res.* **12**, 63.
(6) WOLLMAN & WOLLMAN (1933). *C.R. Soc. Biol., Paris*, **112**, 164.
(7) EVANS (1934). *Publ. Hlth Rep., Wash.*, **49**, 1386.

(*MS. received for publication* 6 *January* 1943)

637.333.1:612.321.6

## 303. THE PRODUCTION OF RENNET FROM LIVING CALVES

BY N. J. BERRIDGE,* J. G. DAVIS, P. M. KON, S. K. KON AND F. R. SPRATLING,
*National Institute for Research in Dairying, University of Reading*

(With 8 Figures)

### INTRODUCTION

Fomin (1) has suggested that the production of rennet from live calves is more economical than the use of dried vells from the slaughtered animals. While this was probably true under the conditions then prevalent in Russia, it is less likely to hold in this country where labour and other costs are greater. Under war-time conditions, however, the possibility that abomasa or vells will become unobtainable for rennet manufacture has to be considered. The short investigation recorded here was carried out to examine the practicability and the economic aspects of Fomin's method.

Fomin obtained abomasal juice containing rennin by allowing calves to drink diluted whey and removing the liquid from the abomasum through a fistula fitted with an ebonite cannula. We also found this method to be successful.

* Benger Research Fellow.

### Operation

The technique used was a modification of the method described by Phillipson & Innes (2) for making permanent fistulae in the abomasa of sheep by the use of ebonite cannulae. Certain alterations in technique were of course necessary when operating on calves. The two animals used in this experiment were bull calves which had been suckling for the first 4 days of life and were then pail fed. They were operated on when 14 days old. The evening before the day of operation the usual feed was withheld and the following morning 100 g. glucose in water were given. The site of operation was clipped, shaved, washed and immediately before operating swabbed with spirit. Chloroform anaesthesia was induced by inhalation and the calf restrained on its left side on the operating table. The usual aseptic precautions were taken.

An incision 4–5 in. long was made antero-posteriorly from immediately behind the xyphoid cartilage and lying 1 in. to the right of the mid-line. Skin, subcutaneous tissue, muscle and peritoneum were incised and the abomasum was found lying directly below the incision. Part of the greater curvature of the abomasum was drawn through the wound and held in position by bowel forceps. A purse-string suture, diameter about 1 in., was made in the wall of the abomasum, using catgut and care being taken to go only through the serous and muscular coats. The abomasal wall was then grasped at the centre of the circle made by the suture and a piece cut out with scissors, making a hole large enough to allow the inner flange of the cannula to pass through into the lumen of the abomasum. Considerable haemorrhage from the very vascular abomasum was encountered at this point, but no attempt was made to control it. The cannula was inserted into the wound thus made and the purse-string suture drawn tight so that the serous surface of the abomasum was in contact with the stem of the cannula and the wound invaginated. Drawing the suture tight stopped the haemorrhage. The abomasum was then returned to the abdominal cavity. A stab wound was next made through the abdominal wall about ½ in. laterally from the original incision, using a trocar ½ in. in diameter and thrusting from within outwards. This wound was enlarged with scissors and the stem of the cannula was then forced through from the peritoneum to the skin. The original wound was closed by two layers of sutures. The deep layer consisted of interrupted catgut sutures taking in the peritoneum and muscle. The skin was closed by interrupted silk sutures. The cannula was then fixed in position by drawing it through the stab wound to a suitable length, screwing on the outer flange and locking collar and finally the screw cap. Throughout the operation the cannula was kept plugged with gauze to prevent escape of the stomachial contents. This plug was only removed and the screw cap put on when the cannula was finally *in situ*. A wide cotton bandage was pinned round the calf's abdomen to keep the wound clean and the animal was removed from the operating table and allowed to recover consciousness in its pen.

Before passing on to the post-operative treatment, we would like to emphasize here the value of making a stab wound for the accommodation of the cannula away from the original incision—a technique evolved and described by Phillipson & Innes. The advantages of this method are that the healing of the operation wound is not hampered by the irritating presence of the cannula, and conversely, the firm position of the cannula is not affected should any of the sutures of the operation give way.

The cannula used was of ebonite and similar to that described by Phillipson & Innes but with modifications which were necessary for use in calves. A scale drawing of the cannula and a photograph of it in position are given in Figs. 1 and 2.

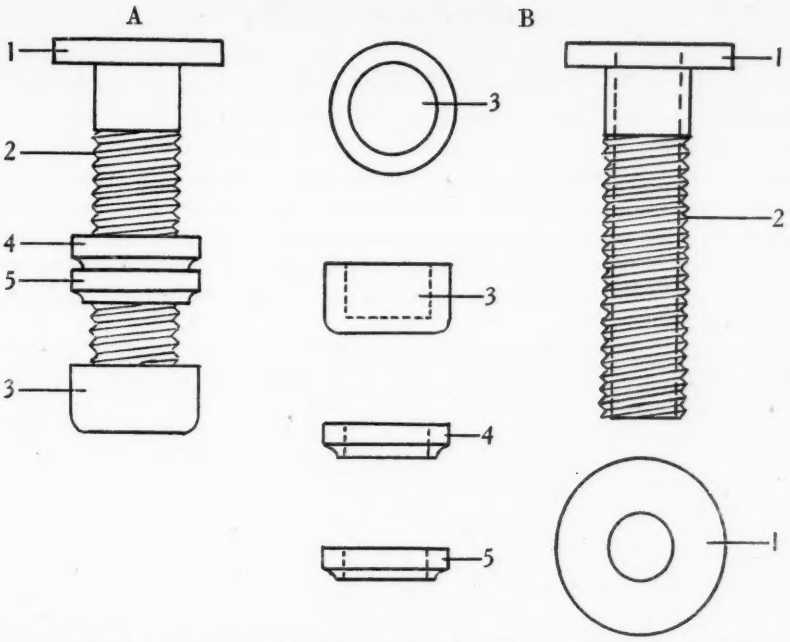


Fig. 1. Cannula—full size. A, assembled; B, in pieces. 1, flange which lies in umen of abomasum; 2, threaded stem; 3, cap closing external end of cannula; 4 and 5, locking rings, the inner of which lies against the calf's skin.

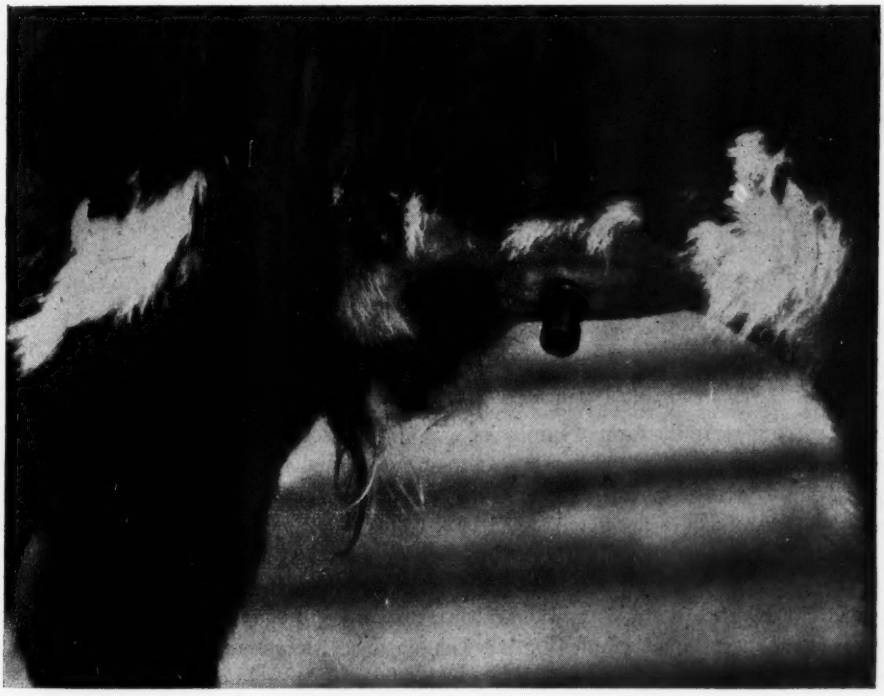

Fig. 2. Cannula in position. Age of calf (no. 1) about 1 month.

### Post-operative treatment

Each calf was seen again in the evening of the operation day and 2½ lb. milk and 100 g. of glucose were given and were taken readily. M and B 693 5 g. was given as a drench the next morning and followed by 2 g. doses on each of the three successive mornings. Recovery was quite uneventful in both calves, and the operation wound healed by first intention. In each case, however, some induration and slight pus formation developed round the cannula. This was dressed daily with acriflavin or Dettol and never attained troublesome proportions. Both calves were slowly brought on to their maximum consumption of milk.

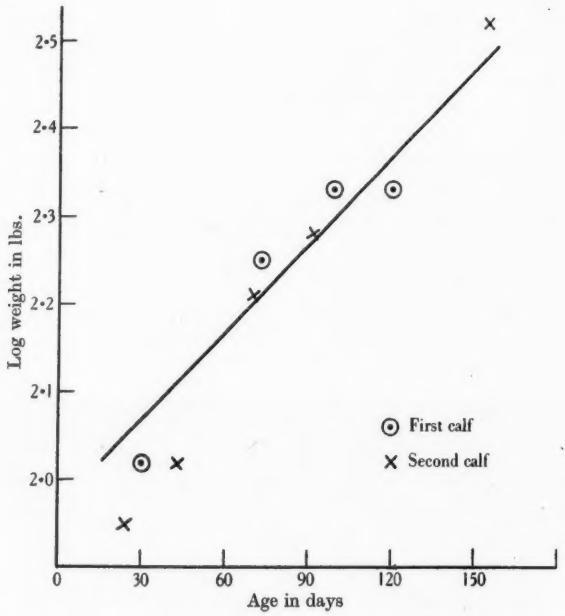


Fig. 3. Rate of growth of experimental calves. The straight line shows the normal growth rate. (Taken from Bartlett & Jameson(4).)

### Health, nutrition and methods of feeding

Care was taken to ensure the good health of the calves during the experiment. They were fed solely on raw full-cream milk with a daily supplement of 90 mg. iron, 9 mg. copper, 9 mg. manganese in the form of sulphates, and 5 g. magnesium carbonate at the beginning of the experiment, rising with the weight of the animal to double this quantity(3). A weekly dose of 7000 i.u. of vitamin D rising similarly was also given. At the age of 1 month the calves received 14 lb. of milk each daily. This quantity was gradually increased at the rate of 3 lb. per month.

The good condition of both animals throughout the experiment is shown by their growth rates given in Fig. 3 and the healthy appearance of one of the animals is shown in the accompanying photograph (Fig. 4). This good health was maintained until the end of the experiment when both animals were slaughtered and sold as veal.

It was found necessary to muzzle the calves during the whole experiment, since otherwise they ate large quantities of straw from the bedding. Not only did the straw make the removal of whey through the cannula more difficult, but it was feared that solid food might stimulate the secretion of pepsin.

During their period of rennet production the calves were fed twice daily. Various feeding times were tried, and the most convenient were found to be midday and late afternoon. The animals could then be used for rennet production during the morning when the abomasum was empty and the appetite good.

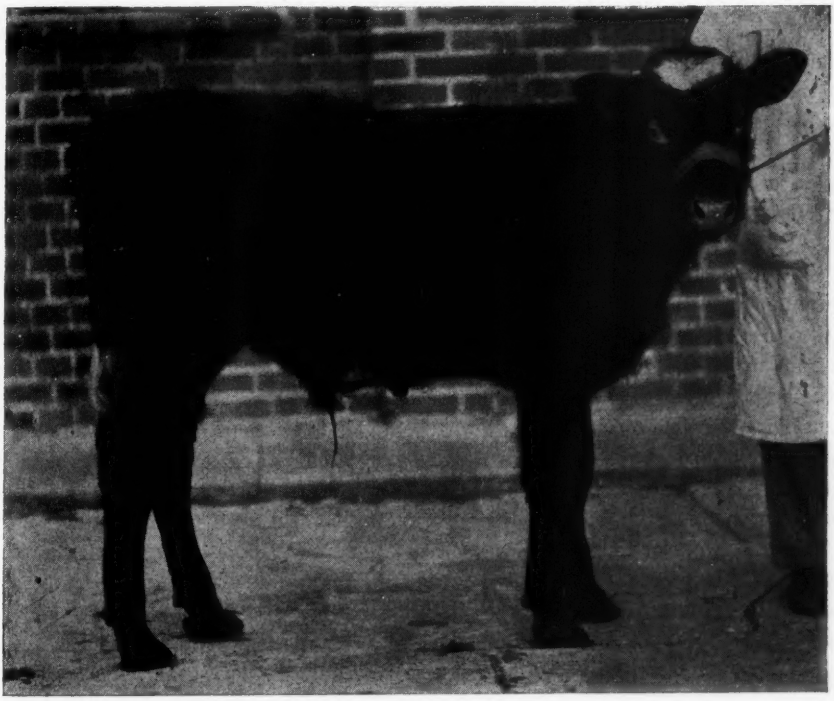

Fig. 4. Calf no. 2 at the age of 3 months. The cannula can just be seen.

Mortenson, Espe & Cannon(5) have shown that 12–18 hr. are required for a calf to digest 2 l. of milk. Thus the only time when the abomasum is empty is early morning. Since an empty abomasum is necessary for this method of obtaining rennet, the only suitable time for carrying out the process is the morning. The animal's appetite is also keenest at this time. Other times for rennet extraction were tried and found to be less satisfactory. On some occasions rapid emptying of the stomach was secured by adding sodium citrate to the previous feed of milk, and on others by treating the milk with trypsin. These methods were successful, but no better than using the early morning for rennet production, and involved more trouble.

### Production of rennet

Abomasal juice was usually obtained by emptying the abomasum through the cannula about half an hour after giving the calves diluted whey.* An alternative method, consisting in feeding the diluted whey by bottle and allowing it to flow out of the abomasum continuously as the liquid was being taken, was tried several times. It had no appreciable effect on the yield of rennet. The bottle was provided with a narrow jet so that a quarter of an hour was taken by the calf to empty it. It was noticed that the liquid coming through first had only a half to a third of the activity of that coming later, so that there is a lag of about 5 min. between stimulation and maximum secretion.

The presence of curds and straw particles in the stomach necessitated the frequent clearing of the cannula during the outflow of the juice. On some occasions not more than a few millilitres of juice would flow after clearing the cannula before it again became clogged, so that at these times about a quarter of an hour was needed to empty the abomasum. For clearing the cannula, a narrow rod having a wider portion at one end, a little smaller in diameter than the inside of the cannula, and a handle at the other, was found to be suitable. It was also effective in preventing the growth of tissue over the inner end of the cannula. This growth occurred if the calves were left undisturbed for several days, and made itself felt by increased resistance to the passage of the rod, and by the appearance of a few drops of blood when the tissue had been torn. The calves gave no sign of pain when the cannula was being cleared in this way.

It appears from Fomin's paper that it is necessary to determine for each calf the best time for which the diluted whey should be left in the abomasum. When this was done with these two calves there was found to be no correlation between the total yield of rennet and the time for which whey was left in the abomasum. Even the flow-through method described above gave good yields. The volume of recovered liquid diminished, however, as the time was increased, but the variations from day to day were greater than the variations in one day due to different times of leaving the whey in the abomasum.

Half an hour was found to be a satisfactory time to wait before emptying the abomasum, since with this interval the fluid was not too dilute, and was of such a volume that the loss of a little by incomplete emptying did not matter.

The yields of rennet obtained from the two calves, together with details of the volume of fluid given, volume recovered, time of leaving and any special conditions, are recorded in Table 1. Where the time of leaving is not recorded it was between 20 and 50 min. The unit of rennet activity used in the table is the amount of rennet needed to clot 10 ml. of standard substrate in 100 sec. at 37° C. The strength of any rennet solution was therefore obtained by using the formula

$$\text{Activity} = \frac{100d}{CT},$$

where $d$ is the dilution of the original solution when 1 ml. of the dilution is added to 10 ml. of substrate, or is the volume in ml. of the original solution added to 10 ml. of substrate when the solution is used without dilution, and where $CT$ is the clotting time in seconds, at 37° C.

An exact value can be obtained for the clotting time by having a glass rod in the test-tube containing the milk and enzyme, and making with it a thin translucent film of milk on the wall of the tube. When the milk clots, the film breaks into visible 'flocs'. The change occupies less than 1 % of the total clotting time.

* 1 pt. whey to 3 pts. warm water.

The standard substrate was made by dissolving spray-dried skim-milk powder in $N/50$ calcium chloride solution at the rate of 12 g./100 ml., using a plunger and barrel-type egg-whisk. Good quality milk powder which had been dried at a low temperature was used, and since all measurements were made with powder from the same batch the unit was constant. Tests were carried out from time to time to ensure that this was so. With care, and by carrying out determinations in triplicate, mean values can be obtained which do not differ by more than 2 % from one another. For these experiments, however, such accuracy was not necessary and replicate determinations were not carried out. The figures may therefore deviate by ±5 % from the true value.

Table 1.

| Age of calf days | Volume of whey given l. | Time left in abomasum min. | Volume recovered l. | Activity rennet units/ml. | Yield (activity × volume recovered) thousands of units | Remarks |
|---|---|---|---|---|---|---|
| | | | *Yields of rennet from the first calf* | | | |
| 20 | 1·0 | 30 | 0·40 | 3·5 | 1·4 | |
| 21 | 1·5 | 30 | 0·60 | 5·0 | 3·0 | |
| 22 | 1·5 | 20 | 0·58 | 5·5 | 3·2 | |
| 25 | — | — | 0·70 | 3·5 | 2·4 | Whey given by bottle |
| | 1·3 | — | 0·35 | 6·0 | 2·1 | Do. |
| | 1·6 | — | 0·92 | 2·3 | 2·1 | Do. |
| 26 | 1·5 | — | 0·80 | 2·7 | 2·2 | Do. |
| 27 | 1·5 | — | 0·77 | 3·2 | 2·5 | Do. |
| 30 | 1·5 | 30 | 0·28 | 4·5 | 1·3 | Low volume recovery due to curds and straw in abomasum |
| | 1·5 | 30 | 0·10 | 8·4 | 0·8 | Do. |
| | 1·5 | 20 | 0·15 | 3·6 | 0·5 | Do. |
| 31 | 1·5 | — | 0·32 | 6·0 | 1·9 | |
| | 1·5 | — | 0·36 | — | — | |
| 32 | — | — | — | — | — | Castor oil given to remove bowel obstruction |
| 38 | 1·5 | 30 | 0·30 | 6·3 | 1·9 | This day's yields were obtained in the afternoon. The morning feed* contained 6 g. sodium citrate to prevent curd formation. The stomach was empty by early afternoon. The 30 min. after 0 volume given means 30 min. after the previous extraction |
| | 2·0 | 0 | 1·00 | 2·6 | 2·6 | |
| | 0·0 | 30 | 0·06 | 20·0 | 1·2 | |
| | 2·0 | 30 | 0·60 | 5·0 | 3·0 | |
| 46 | 2·0 | 30 | 0·32 | 6·0 | 1·9 | Sod. cit. again given but not effective this time |
| 47 | 2·0 | — | 0·87 | 4·7 | 7·4 | Milk treated with trypsin given for morning feed* |
| | 2·0 | — | 0·70 | | | |
| 48 | 2·0 | — | 0·36 | | | |
| | 2·0 | — | 0·58 | 6·25 | 9·7 | Do. |
| | 2·0 | — | 0·60 | | | |
| 51 | 2·0 | — | 0·92 | | | |
| | 2·0 | — | 0·57 | 4·5 | 11·5 | No morning feed* |
| | 2·0 | — | 0·47 | | | |
| | 2·0 | — | 0·60 | | | |
| 52 | 2·5 | — | 1·29 | | | |
| | 2·0 | — | 0·78 | 4·5 | 13·5 | Plain milk for morning feed* |
| | 2·0 | — | 0·92 | | | |
| 53 | 2·5 | — | 1·15 | | | |
| | 2·5 | — | 1·28 | 4·3 | 13·6 | Plain milk for morning feed* |
| | 2·5 | — | 0·73 | | | |
| | 2·5 | — | 0·60 | 5·0 | 6·6 | Physiological saline flavoured with diacetyl was used for these two 'perfusions'. It was not taken readily |
| | 2·5 | — | 0·73 | | | |
| 55 | 2·5 | — | 1·05 | 5·5 | 12·0 | |
| | 2·5 | — | 1·13 | | | |
| 73 | 2·5 | — | 0·50 | 11·0 | 7·9 | These were obtained before the morning feed* |
| | 2·5 | — | 0·22 | | | |
| 74 | 2·5 | 40 | 0·70 | 7·0 | 4·9 | Abomasum apparently full of solids. Belly swollen. Appetite very poor |
| | 1·0 | — | 0·13 | 10·0 | 1·3 | Obtained before morning feed* |
| 90 | 2·5 | — | 0·88 | 6·0 | 5·3 | |
| 120 | — | — | — | — | — | Cannula came out. Calf slaughtered next day |

* 'Feed'—a meal which the calf was allowed to digest and absorb.

Table 1 (*continued*)

| Age of calf days | Volume of whey given l. | Time left in abomasum min. | Volume recovered l. | Activity rennet units/ml. | Yield (activity × volume recovered) thousands of units | Remarks |
|---|---|---|---|---|---|---|
| | | | *Yields of rennet from the second calf* | | | |
| | *Note.* On some days the fluid recovered from several sham feeds was bulked and only the total yield determined. In such cases the number of portions given and their volume are indicated by the appropriate figures and the multiplication sign; for example, 4 × 2 on the calf's 47th day. | | | | | |
| 28 | 1·5 | 75 | 0 | — | — | Clot or skin over end of cannula prevents fluid flowing except when clearing rod is in position. Activities are the maximum values obtained after activation at $pH_2$ |
| | 1·5 | 30 | 1·15 | 12·0 | 13·8 | |
| | 1·5 | 45 | 0·75 | 11·5 | 9·0 | |
| 31 | 1·5 | 35 | 0·30 | 5·9 | 1·7 | Activities determined after standing 1 day at room temp. |
| | 1·5 | 50 | 0·18 | 16·5 | 2·9 | |
| | 1·5 | 28 | 0·50 | 5·6 | 2·8 | |
| 38 | 2. | 20 | 1·70 | 5 | 8·5 | Activity after 1 day |
| | 2 | 35 | 0·80 | 11·6 | 9·2 | Activity after 2 days |
| | 2 | 40 | 0·70 | 11·9 | 8·3 | Do. |
| 40 | 2 | 20 | 1·40 | 7·1 | 9·9 | |
| | 2 | 40 | 0·90 | 10·9 | 9·8 | |
| | 2 | 30 | 0·80 | 7·1 | 5·7 | |
| | 2 | 20 | 1·20 | 3·8 | 4·6 | |
| 41 | 2 | 40 | 0·78 | 7·7 | 6·0 | |
| | 2 | 40 | 0·72 | 10 | 7·2 | |
| | 2 | 40 | 0·68 | 8·5 | 5·8 | |
| | 2 | 40 | 0·55 | 9·4 | 5·2 | |
| 42 | 2 | 40 | 2·00 | 4·8 | 9·6 | |
| | 2 | 55 | 0·70 | 9·1 | 6·4 | |
| | 2 | 38 | 0·95 | 5·7 | 5·4 | |
| | 2 | 30 | 0·80 | 5·4 | 4·3 | |
| 45 | 2 | 65 | 0·25 | 8·3 | 2·1 | |
| | 2 | 55 | 0·40 | 7·4 | 3·0 | |
| | 2 | 11 | 1·40 | 1·9 | 2·7 | |
| | 2 | 51 | 0·20 | 9·8 | 2·0 | |
| 46 | 2 | 50 | 0·50 | 6·7 | 3·3 | |
| | 2 | 50 | 0·50 | 9·1 | 4·5 | |
| | 2 | 30 | 0·40 | 10·6 | 4·2 | |
| | Now started rennet production in morning and feeding at midday and late afternoon | | | | | |
| 47 | 4 × 2 | — | 2·80 | 5·9 | 16·5 | |
| 48 | 2 | — | 1·10 | 4·6 | 5·1 | |
| | 3 | — | 0·94 | 5·5 | 5·2 | |
| | 3 | — | 1·30 | 3·2 | 4·2 | |
| | Started giving sodium chloride to replace chloride lost in gastric juice | | | | | |
| 49 | 4 × 3 | — | 3·70 | 4·7 | 17·4 | |
| 50 | 4 × 3 | — | 5·00 | 4·8 | 24 | |
| 52 | 4 × 3 | — | 3·70 | 5·8 | 21·5 | |
| 53 | 3 | — | 1·00 | 3·4 | 3·4 | |
| | 3 | — | 0·70 | 7·7 | 5·4 | |
| | 3 | — | 0·90 | 4·7 | 4·2 | |
| 54 | 3 | — | 1·10 | 2·4 | 2·6 | |
| | 3 | — | 1·40 | 2·7 | 3·8 | |
| 55 | 4 × 3 | — | 6·00 | 4·1 | 24·6 | |
| 56 | 4 × 3 | — | 4·00 | 7·5 | 30·0 | |
| 60 | 4 × 3 | — | 4·50 | 7·2 | 32·4 | |
| 61 | 4 × 3 | — | 3·70 | 6·7 | 24·8 | |
| 66 | 4 × 3 | — | 4·50 | 4·0 | 18·0 | |
| 67 | 3 × 3 | — | 1·70 | 2·4 | 4·1 | Diluted milk instead of diluted whey |
| 68 | 2 × 3 | — | 2·00 | 3·6 | 7·2 | |
| 69 | 3 | — | 2·30 | 6·6 | 15·2 | |
| 70 | 3 | — | 2·00 | 5·8 | 11·6 | |
| 71 | 3 | — | 0·50 | 8·1 | 4·0 | |
| 73 | 3 | — | 0·70 | 1 | — | |
| 74 | 3 | — | 0·40 | 4·9 | 1·8 | From the 69th to the 74th day the calf was without a muzzle. This accounts for the low volumes and for the poor appetite causing only one lot to be taken. The abomasal juice contained a large quantity of straw |
| 75 | 2 × 3 | — | 2·20 | 9·0 | 19·8 | |
| 76 | 2 × 3 | — | 3·80 | 4·0 | 15·2 | |
| 77 | — | — | — | — | — | Refused to drink any whey |

This calf was then gradually placed on a diet of solid food, and pepsin production observed (see later).

Table 1 may be summarized by giving the mean yield of rennet for each 'perfusion' and its standard deviation. These are 3120 units and ±1330 for the first calf and 5680 units and ±2560 for the second one.

Thus it is clear that the yields of rennet per sham feed were very variable. The spot diagrams (Figs. 5 and 6) show that there was a definite increase with age in yields from the first calf, while no increase can be detected with the second. This may be connected with the fact that the first animal was often allowed to rest, while the second was given the diluted whey as often as he would drink it, to obtain an idea of the maximum possible yield as well as the yield per feed.

On the 67th day milk diluted with 5 vol. of water was used instead of whey for the second calf. Although its better flavour would be expected to stimulate secretion, it was found to be less satisfactory, for both the volume and the activity of the abomasal juice were low, and filtration was difficult. The low yield may have been due to the presence of curds in the abomasum hindering the outflow of fluid and preventing the feeling of hunger which usually stimulates secretion. Solid matter in the abomasum usually caused low yields. This and many other factors probably have an effect on the amount of liquid passing into the rumeno-reticular cavity. The functioning of the oesophageal groove is a complex reflex depending on many factors, and its failure may well be the cause of the loss of fluid often experienced (6). The slow filtering was probably due to the presence of albumins and globulins in the solution. When whey was used it was sterilized so that these proteins were coagulated and removed from solution.

### MAINTENANCE OF CHLORIDE BALANCE

It was thought possible that the loss of chloride in the gastric juice removed might be important, so this was ascertained by titrating samples of the juice with silver nitrate and comparing the figures obtained with those for the diluted whey. The low *p*H of the liquid made the titrations a little difficult, but the rough values obtained were sufficient to indicate how much salt it might be necessary to add to the diet. Three samples of gastric juice obtained when the second calf was 48 days old contained 0·26, 0·22 and 0·26 % of chloride expressed as sodium chloride, and one obtained at the age of 42 days contained also 0·26 %. The concentration of chloride expressed as the sodium salt in the diluted whey administered, consisting of 500 ml. sterilized whey and 1500 ml. of tapwater, was 0·088 %. Thus if 2 l. of this liquid are given and 1 l. of abomasal juice is recovered, the loss of salt from the calf is 0·87 g. With four such 'perfusions' per day the daily loss will be 3·48 g. so that a dose of 5 g. of sodium chloride will cover the loss. This quantity of salt was given and did seem to cause a slight improvement in appetite, but the effect was small compared with that of other conditions. The amount of chloride (as NaCl) obtained from the milk given would be between 12 g. and 13 g./day. If the chloride content of the milk had been low, the extra dose of sodium chloride would have been of value to the animal.

### ACTIVATION OF THE PRORENNIN IN ABOMASAL JUICE

Most of the samples of abomasal juice contained very little prorennin, and this could usually be fully activated by allowing the juice to stand at its normal *p*H overnight. (For *p*H values see later section.) These facts are exemplified by the following results:

(1) Two samples of juice from the first calf at the age of 25 days had activities of 3·2

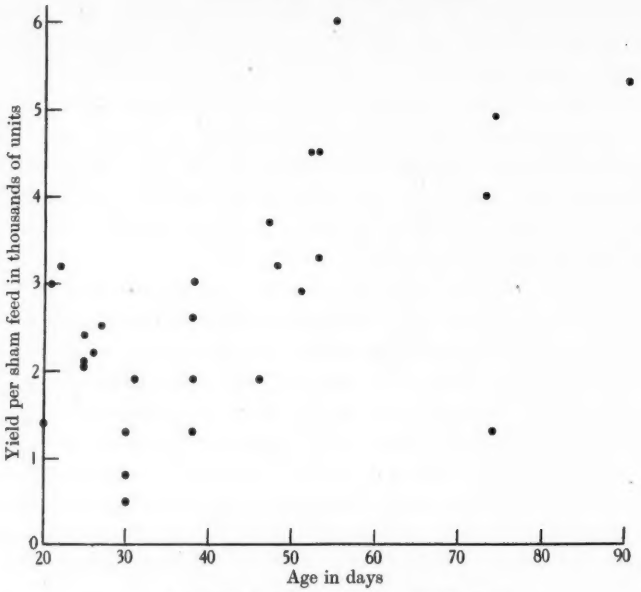


Fig. 5. Yields of rennin from the first calf.

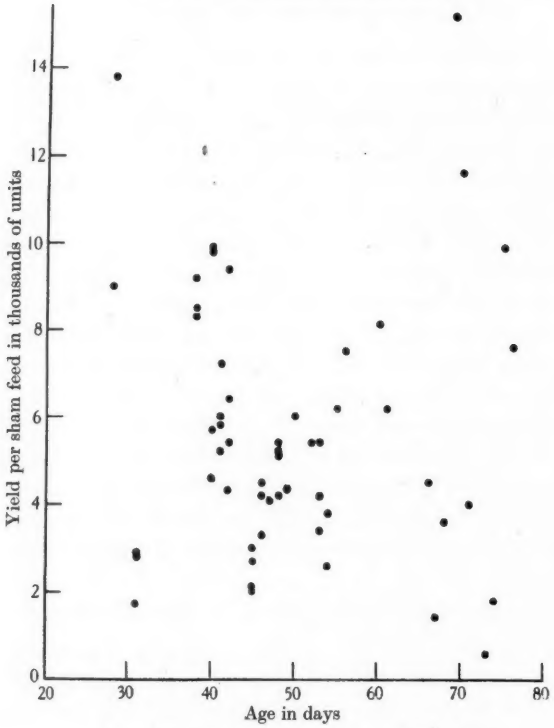


Fig. 6. Yields of rennin from the second calf.

and 5·2 units/ml. respectively. After activation at *p*H 2 overnight these were raised to 3·5 and 6·0.

(2) Two samples obtained from the first calf when it was 28 days old gave the results shown in the following table:

| | | |
|---|---|---|
| Original activity | 4·9 | 8·0 |
| Activity after 24 hr. at room temp., *p*H 4·4 and 3·7 respectively | 6·0 | 10·0 |
| Activity after 1 hr. at *p*H 2 | 12·5 | 11·0 |
| Activity after 24 hr. at *p*H 2 | 7·0 | 11·5 |

(3) At the age of 38 days this calf gave three samples, the *p*H values of which were 3·4, 3·1 and 3·0. After a day at room temperature the activities of these samples were 5, 10·4 and 11·6 units/ml., and after a further day their activities were 4·4, 11·6 and 11·9 respectively.

(4) Two days later four samples were obtained which gave the following values:

| | | | | |
|---|---|---|---|---|
| *p*H | 3·1 | 3·0 | 3·1 | 3·5 |
| Activity on day of extraction | 6·5 | 10·9 | 6·6 | 3·8 |
| Activity after 2 days at room temp. | 7·1 | 10·0 | 7·1 | 3·8 |

(5) The total amount of rennin in the bulked liquid from four 'perfusions' carried out on the 42nd day increased on standing at room temperature for a day from 25·3 thousand to 29 thousand units.

(6) When 45 days old the second calf gave four samples of juice, the activities of which increased as shown below, on standing at room temperature:

| | | | | |
|---|---|---|---|---|
| Activity on day of extraction | 7·4 | 6·8 | 1·9 | 9·3 |
| Activity after 1 day | 8·3 | 7·4 | — | 9·8 |
| Activity after 2 days | 7·4 | 6·5 | — | 9·6 |

(7) The following figures were given by three samples obtained on this calf's 53rd day:

| | | | |
|---|---|---|---|
| *p*H | 3·1 | 2·8 | 3·0 |
| Activity on day of extraction | 3·4 | 7·7 | 4·7 |
| Activity after 1 day at room temp. and 1 day in refrigerator | 3·2 | 6·6 | 4·7 |

Thus it is clear that the abomasal juice was usually sufficiently acid to activate all the prorennin in about a day. Accordingly, measurements of activity were usually made after keeping the juice for this time. Where more than one activation method or time was used, the maximum activity has been recorded.

## THE PROTEOLYTIC ACTIVITY OF ABOMASAL JUICE

Pepsin in the juice was determined by a method almost identical with that suggested by Anson (7). The differences were that a dried haemoglobin preparation was used, and that the enzyme was allowed to act at 37° C. The figures obtained cannot therefore be expressed in the $[PU]^{Hb}$ units of Anson and Mirsky, but the unit used is of the same order, and is calculated in the same way.

The figures obtained for secretions of the first calf at the age of 38 days were 0·00019, 0·00007, 0·00016, 0·00019 approximate $[PU]^{Hb}$ units/ml. of juice, giving ratios of rennin to pepsin (in arbitrary units) of 34,500, 39,000, 124,500 and 25,250:1 respectively. The ratio for a commercial rennet was 102,000:1, and for a crude extract of dried abomasa it was 15,000:1. The peptic activity of abomasal juice from the same calf at the age of 90 days was 0·002 and the ratio 3,105:1. Thus there was a ten-fold increase in the amount of pepsin secreted, while the milk-clotting activity did not increase much.

More extensive experiments were carried out with the abomasal juice from the second calf. The *p*H-proteolytic activity curves for a number of samples were determined. In the first experiment portions of a 2 % haemoglobin solution were adjusted to *p*H values of 2, 3, 4 and 5, using the glass electrode. To 1 ml. of this solution 0·2 ml. of abomasal juice was added, and after 15 min. at 37° C., precipitation with trichloracetic acid and colour development followed as in the method of Anson and Mirsky. The activities in the same units as before, but with each figure multiplied by $10^4$, were 6·1, 9·4, 9·7 and 4·5, the blank value being about 3. Thus there is a maximum activity between *p*H 3 and 4.

A second experiment was run in a similar manner with the same juice, but using solutions of *p*H values from 3 to 7. The *p*H values were redetermined after a few hours to ensure that no change had taken place. Digestion blanks using boiled enzyme were run at each *p*H. The results are shown in Table 2. A digestion period of 10 min. was used.

Table 2. *Proteolytic activity of abomasal juice at various p*H *values*

| *p*H | 3 | 3·2 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| Activity × $10^4$ | 5·7 | 5·2 | 6·5 | 2·2 | 0 | 0 |

No activity was obtained at *p*H 6. In order to obtain more detailed results, the experiment was repeated, and amounts of acid needed to give *p*H values of approximately 1·5, 2, 2·5, 3, 3·5, 4 and 4·5 were added to portions of the haemoglobin solution, and the mixtures left in the refrigerator for 24 hr. for equilibrium to become established. The actual *p*H values were then measured, and the solutions used as already described. The results are shown in Table 3. Table 3 also shows the results for a second sample of juice obtained on the same day (calf 53 days old) and for a sample of commercial rennet.

Table 3. *Proteolytic activities at various p*H *values*

| | Activities × $10^4$ | | |
|---|---|---|---|
| *p*H | Juice 1 | Juice 2 | Commercial rennet |
| 1·50 | 0·9 | 0·4 | 14 |
| 1·79 | 1·5 | 0·5 | 22 |
| 2·26 | 3·3 (2·7) | 1·3 | 41 |
| 3·10 | 5·0 (2·8) | 2·0 | 45 |
| 3·76 | 2·8 | 1·4 | 30 |
| 4·06 | 3·0 | 1·3 | 26 |
| 4·24 | 2·2 | 1·1 | 15 |

The third and fourth values in column 2 appeared to be too high and were therefore repeated, giving the values in brackets. The curves obtained by plotting these three sets of figures are shown in Fig. 7.

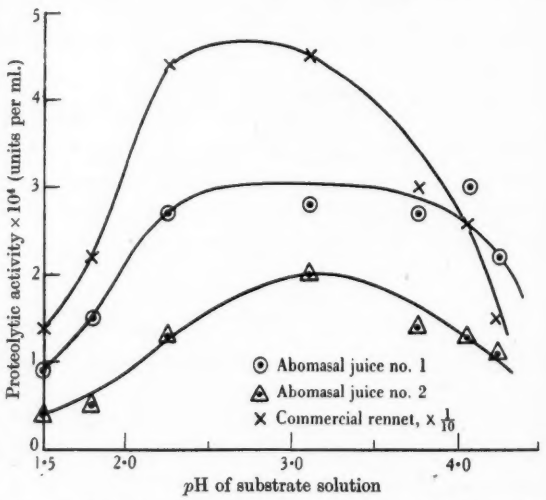


Fig. 7. Proteolytic activity of abomasal juice and of commercial rennet at various *p*H values.

## Production of pepsin

In order to ascertain how the gastric secretion changes as a calf is taken off milk diet, *p*H-activity curves were determined during the change. Three samples of juice were obtained from the second calf; the first when the animal was about 3½ months old and

had been given 2 lb. of meal on 2 days, a week before the sample was taken, the second 10 days later and 10 days after changing from milk had commenced, and the third 20 days after this, when the change was complete.

The haemoglobin solutions were preserved at 0° C., and the same stock used for each juice. The *p*H values were found to have altered during the long period of keeping, and the altered values are recorded against the activities of the third juice. (See Tables 4 and 5.)

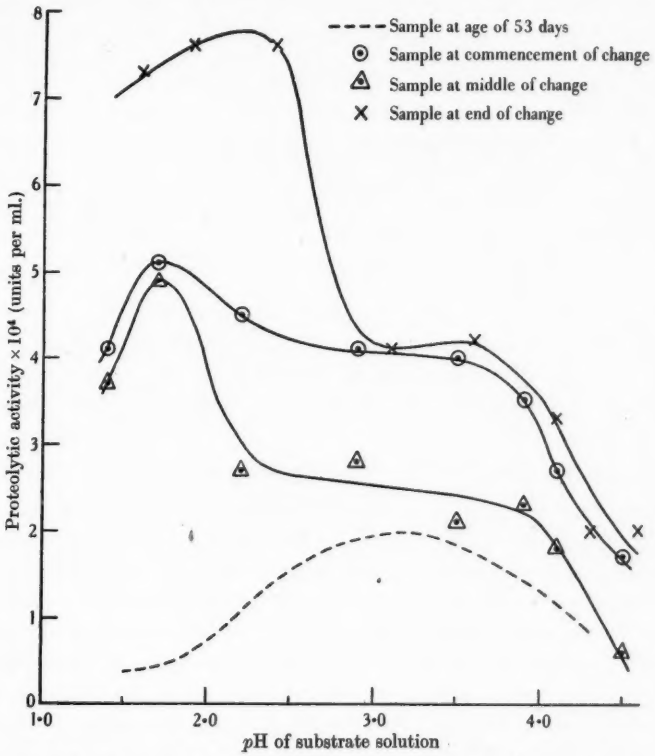


Fig. 8. Development of pepsin in abomasal juice on changing to solid food.

Table 4. *Proteolytic activities of abomasal juice at different pH values during weaning*

| | Activities × $10^4$ | |
|---|---|---|
| *p*H | Sample 1 | Sample 2 |
| 1·4 | 4·1 | 3·7 |
| 1·7 | 5·1 | 4·9 |
| 2·2 | 4·5 | 2·7 |
| 2·9 | 4·1 | 2·7 |
| 3·5 | 4·0 | 2·1 |
| 3·9 | 3·5 | 2·3 |
| 4·1 | 2·7 | 1·8 |
| 4·5 | 1·7 | 0·6 |

Table 5. *Proteolytic activities of abomasal juice at different pH values after weaning*

| *p*H | Activity × $10^4$ |
|---|---|
| 1·6 | 7·3 |
| 1·9 | 7·6 |
| 2·4 | 7·6 |
| 3·1 | 4·1 |
| 3·6 | 4·2 |
| 4·1 | 3·3 |
| 4·3 | 2·0 |
| 4·6 | 2·0 |

Graphs of these figures are shown in Fig. 8. It can be seen that the activity above *p*H 3 remains about the same, while that at the lower *p*H values increases considerably. While these figures are insufficient to prove the point, they do suggest that the food of a calf

is as important as its age in determining the character of its abomasal secretion. It is to be noted that this calf, when taken off a milk diet, was older than are non-experimental animals, and that he was secreting little pepsin at an age when other calves are living solely on solid food. Nevertheless, when solid food was given the production of pepsin rapidly increased.

### The acidity of abomasal juice

The acidity of the juice increased slowly during the first few weeks of the calf's life, and then remained practically constant until weaning commenced, when it increased rapidly. Only two figures were obtained from the first calf, but a larger number from the second. They are shown in Table 6.

Table 6. *p*H *values of abomasal juice*

| Age of calf days | No. of samples measured | *p*H values |
|---|---|---|
| | First calf | |
| About 40 | 1 | About 4 |
| 73 | Mixture of 3 | 2·5 |
| 90 | 1 | 2·9 |
| | Second calf | |
| 28 | 2 | 4·5, 3·7 |
| 38 | 3 | 3·4, 3·1, 3·0 |
| 53 | 3 | 3·1, 2·8, 3·0 |
| 66 | Mixture of 4 | 3·2 |
| 125 | 1 | 2·6 |

### Preparation of concentrated rennet from abomasal juice

The fluid obtained from the calves was a turbid sour-smelling liquid of a grey, brown or red colour, depending upon whether whey proteins, straw, or blood was present. When the calves were young it contained a considerable quantity of coagulated protein with bubbles of entrained gas which caused it to rise to the surface of the liquid as a scum along with any fat which had been present in the whey, or which had remained in the stomach from the previous feed. When the animals became older this scum disappeared, possibly owing to an increase in protein digesting power. It was very difficult to prevent the calves from eating straw, so that the fluid also contained masticated straw, sometimes in such quantity that the sediment which formed on allowing the liquid to stand occupied about half the total volume. The usual volume of the sediment was about a fifth of the total.

The first stage in concentrating the rennet consisted in filtering. This was surprisingly easy to carry out considering the nature of the liquid. A day's yield from one animal would filter over-night, giving a bright yellow filtrate. It was found that leaving the filtration till after the rennet had been concentrated resulted in very great losses, owing to the retention of the major portion of the highly active liquid by the insoluble residue.

A method of precipitating and drying the rennet similar to that described by Fomin was found to be satisfactory, although some loss occurred. Thus 12–13 % of the activity was lost on precipitation and filtration, and a further 20 % on drying. Suspending the moist precipitate in a little water and bringing the *p*H to between 5 and 6 caused the protein to dissolve, giving a highly active solution. There was again a 12 % loss, but the drying loss was avoided. When Fomin's method was closely followed and the moist precipitate was redissolved, a loss of 11 % occurred, but the activity of the liquid thus

obtained diminished, so that after 2 days the total loss was over 26 %. The loss incurred in this process appears to depend to some extent on the time during which the precipitate is exposed to air, being a minimum when it is dissolved immediately filtration is complete, and a maximum when it is dried.

The precipitate obtained from abomasal juice will not dissolve to a sufficiently high concentration in a solution containing more than 5 % of sodium chloride. The usual concentration of this salt in commercial rennets is 15–20 %, this amount being thought necessary to preserve the enzyme. Thus liquid rennets of the usual composition cannot easily be prepared from abomasal juice, but it is possible that the solution would maintain its activity just as well with only 5 % salt. When a concentrated solution of this fistula rennet containing 16 % sodium chloride, and therefore having some of the protein in the form of a suspension, was kept for some time, it was found that its activity diminished at about three times the rate at which ordinary liquid rennet loses activity under the same conditions. Although the precipitate present at this concentration of salt consists of only a small proportion of the total protein, it adsorbs about three-quarters of the rennin. It is not known whether this condition has any effect on the stability.

### CHEESE-MAKING PROPERTIES OF FISTULA RENNET

Two 10 lb. Cheddar cheese were made from rennet obtained from the second calf concentrated in the manner already described. In each case 20 gal. of milk were halved and a cheese made from each half, using exactly the same technique and conditions but with commercial rennet for one and fistula rennet for the other. This was done twice so that the fistula rennet could be compared with two brands of commercial rennet. Throughout the cheese-making process the cheese made from fistula rennet were indistinguishable from the other. The analyses of the cheese and the report of expert tasters are given below. They show that cheese made from the fistula rennet did not differ appreciably from those made with commercial rennet.

Table 7. *Analyses of the cheese*

| | Milk A | | Milk B | |
|---|---|---|---|---|
| | Fistula rennet | Commercial rennet | Fistula rennet | Commercial rennet |
| Percentage moisture | 35·50 | 35·90 | 36·00 | 36·00 |
| Total nitrogen | 3·74 | 3·74 | 3·96 | 3·94 |
| Non-protein nitrogen | 0·74 | 0·78 | 0·76 | 0·78 |
| Protein nitrogen | 3·00 | 2·96 | 3·20 | 3·16 |
| Total Sorensen nitrogen | 0·26 | 0·25 | 0·23 | 0·25 |
| Non-protein Sorensen nitrogen | 0·21 | 0·20 | 0·18 | 0·22 |
| Acidity, as lactic acid | 1·45 | 1·51 | 1·50 | 1·59 |

It is clear that the analytical differences between the two sorts of cheese were insignificant.

Table 8. *Cheese-grading results*

| | Grader D.V.D. | | |
|---|---|---|---|
| | Flavour | Texture | Body |
| 337 Cheese made with fistula rennet | Immature, slightly acid | Fairly close | Sticky |
| 338 Control made with commercial rennet | Slight tang | Fairly close | Good, on weak side |
| 339 Cheese made with fistula rennet | Off | Open | Fair |
| 340 Control made with commercial rennet | Immature, slightly acid, trace of bitterness | Fairly close | Good, but on weak side |

Table 8 (*continued*)

| | Grader V.E.C. | | |
|---|---|---|---|
| | Flavour | Texture | Body |
| 337 Cheese made with fistula rennet | Mild, fairly good | Close | Fairly resilient but with a sticky tendency |
| 338 Control made with commercial rennet | Good | Closer than 339 | Fairly good, softer than 339 |
| 339 Cheese made with fistula rennet | Slightly off and strong | Rather open | Good |
| 340 Control made with commercial rennet | Slightly off and strong | Slightly open | Good |

| | Grader A.S. | | |
|---|---|---|---|
| | Flavour | Texture | Body |
| 337 Cheese made with fistula rennet | Mild, fairly good | Good, close | Good but sticky |
| 338 Control made with commercial rennet | Good | Less open than 339 | Good but softer than 339 |
| 339 Cheese made with fistula rennet | Edible, slightly off | Open | Good |
| 340 Control made with commercial rennet | Edible, but similar to 339 | Slightly open | Good |

## Discussion

There were, of course, too few animals to enable an accurate estimate to be made of the quantities of rennet which could be obtained on a large scale by this means, but since the two calves did not differ considerably from one another, it is probable that the results give a rough idea of the yield that could be expected.

The quality of the rennet left nothing to be desired, but the yield was far too small to make the process a commercial success. A dried abomasum contains 30–40 thousand units and costs at present from 6*d.* to 1*s.* Six perfusions would be needed on the average to get this much from our best calf, and this would take at least a day and a half. The cost of food alone would be considerably more than 1*s.* in this period, since full-cream milk is used. Frequently the emptying of the abomasum takes as long as a quarter of an hour. At least this time would therefore have to be allowed for emptying and giving the next drink of diluted whey in calculating the labour needed on a large scale. Since the abomasum has to be emptied half an hour after feeding and the whole process has to be repeated four times during the half day, one man could not 'work' more than three calves per half day. That is to say, obtaining juice from six calves would be a full time occupation for one man or boy.

In addition to the above considerations there are a number of other difficulties. The frequent attendance of a veterinary surgeon is necessary to watch the health of the animals. Even when this is done there are some occasions when their appetite is poor, so that they will only drink one or two portions of whey, and many occasions when three is the limit. Fomin states that 'perfusion' can be carried out five times daily, but only on one occasion would one of our animals take a fifth portion of liquid, and this was when the third and fourth had been physiological saline instead of diluted whey. The maximum yield possible under our conditions was obtained from the second calf, and in 26 working days this amounted to 429 thousand units. Had he taken four drinks of whey on each day without altering the mean yield per sham feed, the total yield would have been 591 thousand units. Thus just over one-quarter of the 'expected yield' was lost through these 'difficulties'.

Milk was used for feeding in order not to stimulate the secretion of pepsin, but separated milk, supplemented with cheap fat, might be used, and this would reduce the cost of

feeding. Alternatively some still cheaper food might be found which would not cause the secretion of pepsin. The cost of labour might be reduced by devising an automatic method of 'perfusion' so that one man could care for a hundred calves.

Another possibility is that the amount of rennin in the juice might be increased many times by the use of suitable drugs, but considerable work would be needed to find drugs, the continued use of which would not defeat its own end by injuring the health of the animals. Only if these arrangements could be made would the method be suitable for the production of rennet on a commercial scale.

Finally, should dried abomasa become unobtainable, we have, in this method, another means whereby rennet for cheese-making might be obtained.


## SUMMARY

Abomasal juice containing rennin was obtained from living calves by means of an abomasal fistula as described by Fomin, in order to ascertain whether the process could be used as an alternative method of manufacturing rennet, should the present curtailed sources fail completely.

The operation for fistula was performed on the calves at the age of about a fortnight by the technique of Phillipson & Innes. The animals received, during the three months for which the experiment lasted, an exclusive diet of whole milk, supplemented by minerals and vitamin D. Although only two calves were used, we feel that the results are nevertheless representative.

Abomasal juice was obtained by allowing them to drink diluted whey and removing it through the fistula after about half an hour. The mean yield of rennin for each 'perfusion' from the first calf was 3120 units with a standard deviation of $\pm 1330$. The corresponding figures for the second calf were 5680 and $\pm 2560$ respectively. (One unit of rennin clots 10 ml. of substrate in 100 sec. The substrate is made by dissolving 12 g. spray-dried skim-milk powder in 100 ml. $N/50$ calcium chloride solution.)

The abomasal juice of a young calf had a maximum proteolytic activity at *p*H 3–4. Other properties of the juice and the concentrated rennet prepared from it were investigated. Cheese made with the fistula rennet was indistinguishable from the control cheese.

Since dried abomasa contain as much as 30–40 thousand units each, the method of Fomin appears to be too expensive in time, labour and cost of food for use in commercial rennet production in this country.


We gratefully acknowledge the valuable co-operation of Dr N. S. Barron, several members of the staff of the National Institute for Research in Dairying, Mr B. D. Thornley and Benger's Limited, members of the British Dairy Institute staff, and Messrs Cope and Cope, Ltd.

## REFERENCES


(1) FOMIN, D. (1939). *Molochno Maslodel 'naya Prom.* no. 9, p. 16.
(2) PHILLIPSON, A. T. & INNES, J. R. M. (1939). *Quart. J. exp. Physiol.* **24**, 333.
(3) HUFFMAN, C. F., CONLEY, C. L., LIGHTFOOT, C. C. & DUNCAN, C. W. (1941). *J. Nutrit.* **22**, 609.
(4) BARTLETT, S. & JAMESON, J. L. (1932). *J. Dairy Res.* **3**, 310.
(5) MORTENSON, F. N., ESPE, D. L. & CANNON, C. Y. (1935). *J. Dairy Sci.* **28**, 229.
(6) WISE, G. H., ANDERSON, G. W. & MILLER, P. G. (1942). *J. Dairy Sci.* **25**, 529.
(7) ANSON, M. L. (1938). *J. Gen. Physiol.* **22**, 79.


(*MS. received for publication* 4 *December* 1942)

637.143

# 304. THE GAS-PACKING AND STORAGE OF MILK POWDER

By C. H. LEA and T. MORAN, *The Low Temperature Research Station, Cambridge,* and J. A. B. SMITH, *The Hannah Dairy Research Institute, Ayr*

(With 14 Figures)

CONTENTS

Introduction: Types of deterioration in milk powder
Part I. The prevention of tallowiness
The nature of tallowiness:
Possible methods of control
The critical amount of oxygen required for the production of tallowiness
Storage experiments:
Choice of temperature for accelerated tests
Changes in oxygen concentration
Chemical changes in the fat
Summary
Part II. Changes in palatability
Assessment of flavour
Types of flavour
Discussion
Summary
Part III. Prevention of loss of solubility
Introduction
Experimental
Discussion
Summary
Part IV. The technique of inert gas packing
Design of a gas-packing unit
Gas-tightness of the container
Analysis of the headspace gas in cans of milk powder
Packing of roller-dried powders
Packing of spray-dried powders
The effect of compression of milk powders
Discussion
Summary
Part V. Further observations on the gas packing of milk powder, including data on storage for periods exceeding three years
Changes in solubility
Changes in palatability
Change in colour
Density of milk powders
Absorption and desorption of oxygen by milk powders
Keeping properties of gas-packed powder on subsequent exposure to air
Technique of gas-packing
Use of carbon dioxide
Gas-packing in large containers
Discussion
Summary
Acknowledgements
References

## INTRODUCTION

In this country the bulk of the milk powder is manufactured either by the drying of thin films on heated metal drums (roller process) or by spraying into a current of hot air (spray process), the chief difference between the two types of powder being the greater solubility of the spray product in cold water.

The keeping properties of milk powder depend upon the initial quality of the milk and the conditions of manufacture, but in general its storage life in air at ordinary temperatures under the best conditions is of the order of 3–7 months for spray full-cream powder, 6–12 months for roller full-cream powder,* and 12 months or longer for separated powder, after which it becomes progressively less palatable. Attempts have been made to

* In air roller-dried full-cream powders resist the onset of tallowiness for longer periods than do spray-dried powders. In inert gas tallowiness ceases to be the limiting factor and spray-dried powders keep at least as well as roller-dried powders.

extend the storage life of milk powder by packing it in containers filled with inert gas (nitrogen and/or carbon dioxide), and this method has recently been applied to some extent in commercial practice. As far as we are aware, however, no exact scientific work has been published on the principles of the method, and it was hoped that the present investigation would fill this gap.

*Types of deterioration in milk powder*

Owing to the low moisture content of the powder, microbial spoilage will not occur unless water is absorbed by exposure to the air. Milk powder may, however, suffer other forms of deterioration, of which the more important are listed below.

(*a*) *Loss of solubility.* This form of deterioration is considered in Part III of the present communication.

(*b*) *Stale and musty flavours.* The appearance of a stale or musty odour and flavour is a defect liable to occur in both full-cream and separated powders. The fault is believed to be connected with the protein fraction of the milk, and, like loss of solubility, is very much more marked in powders of high moisture content. It is said to be best prevented by protection against air and moisture and by avoiding high or very low (below 0° C.) storage temperatures (1).

(*c*) *Rancid odour and flavour.* The defect usually referred to as 'rancidity' is due to the liberation of free butyric and other volatile fatty acids by the action of lipase originally present in the milk or produced by the growth of micro-organisms prior to drying. It can be avoided by control of the bacteriological quality of the fresh milk, and by exposure of the milk during or prior to drying to a temperature sufficiently high to inactivate lipase. It is claimed that this latter object can be achieved without seriously impairing the solubility of the powder (2).

(*d*) *Fishiness.* Powders of high moisture content occasionally develop fishy flavours on storage owing to the hydrolysis and oxidation of lecithin to trimethylamine (3), or to the oxidation of the unsaturated constituents of the fat in the presence of moisture and of nitrogenous substances (4). Reduction of the moisture content of the powder to below 3·5 % appears to prevent this form of spoilage.

(*e*) *Tallowiness.* The production of unpleasant 'tallowy' odours and flavours by the action of atmospheric oxygen on the milk fat is much the most prevalent and the most important form of spoilage in full-cream powders. Unlike the other defects already enumerated tallowiness cannot be prevented or even reduced by keeping the moisture content of the powder low, in fact very dry powders have been found to develop a tallowy odour and flavour more rapidly and more intensely than powders of normal or of high moisture content (5).

The conclusion to be drawn from the available data is that the prevention of tallowiness is a major problem in the long storage of milk powder. Parts I and II of the present paper deal only with this effect and the methods of overcoming it. Part III discusses changes in the solubility of milk powders on storage, while Part IV deals with the technique of inert gas packing.

## Part I. THE PREVENTION OF TALLOWINESS

C. H. LEA and T. MORAN

### The nature of tallowiness

Tallowy odours and flavours are due to a reaction between fat and oxygen of the atmosphere.

Milk fat, in common with other natural fats, displays a certain initial resistance to oxidation, the reaction proper being preceded by an induction period during which combination with oxygen is slow and the resulting deterioration in flavour slight. The phenomenon of the induction period is considered to be due to the action of traces of protective substances, known as antioxidants or inhibitors, which delay oxidation, and prevent the reaction between oxygen and the fat from attaining its full velocity.

In milk powder the rate of oxidative spoilage of the fat can be influenced by a number of factors. Heat shortens the induction period, increases the rate of oxidation, and shortens the time before the powder becomes tallowy. Even more potent as a factor in inducing tallowiness is light, in particular the ultra-violet and blue rays of the spectrum: exposure to direct sunlight for a few minutes or to diffused daylight for a few hours is sufficient to spoil the powder. Traces of various of the heavy metals, of which copper and (in less degree) iron are the most important from a commercial point of view, accelerate oxidation, and contamination by copper during manufacture tends to limit the storage life of the powder (Table 1). Other factors, perhaps less important and certainly less well understood, are variations in the initial susceptibility of the milk to oxidative taints, the destruction or removal of oxidizing enzymes during processing, stabilization of the fat by the addition of sugar or of other substances, and the harmful effect of age or of poor bacteriological quality of the raw milk in producing a high acidity and possibly also enzymes capable of accelerating oxidation of the fat. Finally, since tallowiness is the result of a reaction involving atmospheric oxygen, exclusion of the latter might be expected to prevent the change.

Table 1. *Effect of copper on the development of tallowiness in milk powder* (6)

| Copper added to the milk (parts per million of milk solids) | Time to become tallowy at room temperature |
|---|---|
| 0 | 12 months |
| 0·7–1·0 | 9–10 months |
| 3–7 | 5–7 months |
| 10–15 | <50 days |

### *Possible methods of control*

Methods aiming at improving the storage life of milk powder by preventing or inhibiting tallowiness fall into three classes, namely:

(*a*) Those aiming at increasing the inherent resistance of the milk to oxidation, by selective breeding of the cows, control of the diet, etc. Very little is yet known concerning the influence of such factors on the keeping properties of liquid milk and of butter fat, and nothing at all concerning their effect on milk powder.

(*b*) Those concerned with improving methods of processing with the object of preserving the natural resistance to oxidation of the fat, e.g. by improvement of the quality and freshness of the raw milk used; by replacement of copper or copper-alloy equipment by stainless steel; by clarification of the milk and perhaps pasteurization at higher temperatures to destroy oxidizing enzymes; by drying *in vacuo* or in nitrogen instead of in air in order to minimize oxidation during drying; by reduction of the moisture content to an optimum figure, probably in the neighbourhood of 2–3 %(5); by rapid cooling after drying, and exclusion of light until the powder has been packed into opaque containers, which should be done with a minimum of delay; by storage at low temperatures, probably just above the freezing-point since it has been stated that this is preferable to storage at freezing temperatures.

(*c*) Methods of packing aiming at reducing the amount of oxygen available for oxidation, e.g. compressing the powder into blocks, or packing in gas-tight containers either *in vacuo* or in inert gas.

In the storage of milk powders in gas-tight containers sealed under vacuum or filled with nitrogen or carbon dioxide it is virtually impossible to remove the last trace of oxygen. Definition of the problem in both the theoretical and practical sense, therefore, consists in determining the effects of different low partial pressures of oxygen on the rate at which tallowiness or other oxidative changes take place.

In our first approach to this problem direct experiments were carried out on fresh, air-free butter fat which was heated with various known amounts of oxygen until the latter had reacted completely with the fat.

### *The critical amount of oxygen required for the production of tallowiness*

About 10 g. of fresh butter fat was weighed into glass tubes of about 40 ml. capacity, heated for 1 hr. at 100° C. with occasional shaking in the vacuum produced by an oil pump (to remove dissolved air) and cooled in a water-bath to 25° C. The calculated quantity of dry air was then admitted and the tube, still immersed in the bath, sealed off. The amounts of oxygen admitted were 0·2, 0·1, 0·05, 0·02 and 0·01 ml. (at N.T.P.) per gram of fat, five or six tubes being filled at each concentration and stored at 100° C. Tubes open to the air at both ends served as 'open' controls, while others filled nearly to the top and flushed with nitrogen and completely exhausted before sealing served as controls for the tasting tests. Tubes were opened at intervals and the contents examined for peroxide value (Fig. 1) and for flavour.

On prolonged heating, even in the absence of oxygen, the flavour of butter fat was found to change, becoming stronger and more like that of butter toffee. Tubes heated *in vacuo* for approximately the same period as the samples under investigation were therefore used as 'controls' in the tasting tests. This change in flavour on heating rendered the detection of very slight deterioration due to oxidation more difficult, but did not interfere with the detection of tallowy flavours of strength 2 or more (Table 2). The experiments indicated that when the amount of oxygen available was less than 0·02 ml./g. of butter fat 'off' flavours produced were slight, though some loss of fresh flavour, which the method used was not capable of detecting, might have occurred. At 0·05 ml./g. the fat showed very definite tallowiness, at 0·1 ml. it had become unpalatable and at 0·2 ml. very unpalatable.

0·01 ml. of oxygen per g. of fat corresponds approximately to 0·12 % of oxygen in the free space of a can of (uncompressed) roller full-cream powder, or 0·23 % in a can of spray full-cream powder (packing 130 and 200 g. respectively into a can of 400 ml. capacity). Obviously, in the case of milk powder, dilution of the fat with nearly three parts of non-fatty material of appreciable flavour might be expected to increase the amount of oxygen per gram of fat necessary to produce detectable spoilage. Furthermore, absorption by non-fatty substances of part of the available oxygen, either directly (as is subsequently shown to occur) or by reduction of the fat peroxide, would increase the apparent amount of oxygen per gram of fat necessary to produce tallowiness. On both

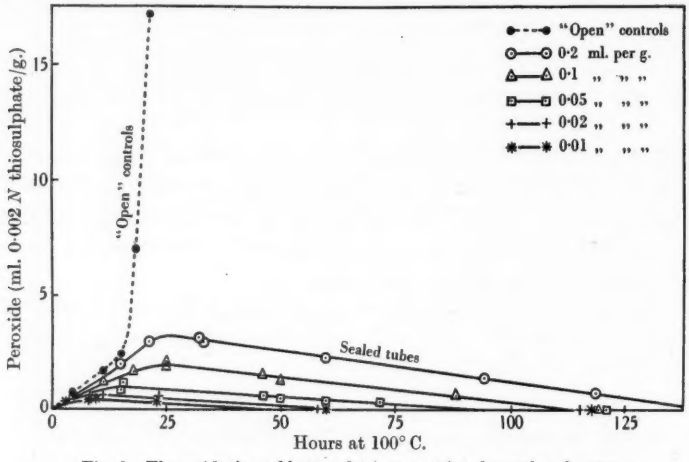


Fig. 1. The oxidation of butter fat in a restricted supply of oxygen.

Table 2. *Palatability of butter fat after heating with oxygen in sealed tubes at* 100° C.

| Oxygen available (ml. at N.T.P. per g. of fat) | Flavour* At approx. maximal peroxide value | | After approx. 50 hr. | | After approx. 129 hr. | |
|---|---|---|---|---|---|---|
| 0·01 | 0·2 | 0·4 | 0·0 | 0·5 | 0·3 | 0·5 |
| 0·02 | 1·0 | 0·7 | 0·5 | 0·7 | 0·7 | 0·8 |
| 0·05 | 1·6 | 2·5 | 2·0 | 2·7 | 2·0 | 2·7 |
| 0·10 | 3·1 | 3·5 | 3·5 | 3·2 | 3·3 | 3·2 |
| 0·20 | 3·6 | — | 4·0 | — | 4·0 | — |

* Flavour scale as on p. 174.

counts therefore it is clear that the concentration of oxygen permissible in the free space in a can of full-cream milk powder will be greater than 0·12–0·23 %. These considerations led us to expect that the critical range of oxygen concentrations might reasonably be 0·5–1·5 %, but only direct experiment could give the concentrations precisely.

## Storage experiments

The general scheme of the experiments was to pack freshly prepared milk powders as expeditiously as practicable† into gas-tight cans in mixtures of nitrogen and oxygen ranging from air (20·8 %)‡ down to 0·1 % oxygen, the latter figure being that which the

† One day after manufacture in case of roller-dried powders and 2 days in case of spray-dried powders.

‡ Under the conditions employed in packing, laboratory air approximated more closely to this figure than to the 20·93 % of the outside atmosphere.

experiments on butter fat had indicated as reasonably certain to protect the fat from talliowiness for an indefinite period. In practice the gas-packing of milk powder was found to be less straightforward than anticipated, and a certain amount of experimental work had to be done before uniform packs with the required low contents of oxygen could be obtained (Part IV). The cans were sealed at atmospheric pressures and the powders packed without compression, since it was felt that the results obtained would correlate keeping properties with the absolute amount of oxygen available per gram of powder, and could therefore be applied also to predict the effect of compressing the powder and of packing *in vacuo* or under reduced pressure. Data concerning the packing of the various powders are given in Table 3.

Table 3. *Density of packing of milk powders used for the storage experiments*

| Type of powder | Packing | Headspace mm. | Weight in 400 ml. can g. | Volume of air in can ml. | ml. air per g. of powder | ml. $O_2$ (at 0° C.) per g. of powder |
|---|---|---|---|---|---|---|
| Roller full-cream(1.2.3) | Loose* | 7–10† | 130 | 299 | 2·30 | 0·446 |
| Roller full-cream(1.2.3) | Loose | None | 143 | 289 | 2·02 | 0·392 |
| Roller full-cream(1.2.3) | Hand compressed | 7–10 | 187 | 255 | 1·36 | 0·264 |
| Roller full-cream(1.2.3) | Hand compressed | None | 206 | 240 | 1·16 | 0·226 |
| Roller full-cream‡ | Loose | 7–10 | 165 | 272 | 1·65 | 0·230 |
| Roller full-cream | Loose | None | 182 | 259 | 1·42 | 0·275 |
| Spray full-cream(1.2) | Loose | 7–10 | 200 | 245 | 1·22 | 0·238 |
| Spray full-cream(1.2) | Loose | None | 220 | 230 | 1·05 | 0·203 |
| Roller separated | Loose | 7–10 | 140 | 303 | 2·16 | 0·419 |
| Roller separated | Loose | None | 154 | 293 | 1·90 | 0·368 |
| Spray separated | Loose | 7–10 | 235 | 237 | 1·01 | 0·196 |
| Spray separated | Loose | None | 258 | 221 | 0·86 | 0·167 |

* Settled by lightly tapping the can, but not otherwise compressed.

† As used in the majority of the storage experiments. ‡ By another manufacturer, not stored.

*Choice of temperature for accelerated tests*

Since it was desired to ascertain the effect on milk powders of storage for several years, it was necessary to employ accelerated ageing tests in order to obtain results within a reasonable time. Preliminary trials on several milk powders indicated that temperatures above 60° C. rapidly produced caramel-like flavours and a certain amount of discoloration, but that 40 or even 50° C. had no such effect.

Two temperatures were therefore chosen for the accelerated tests, namely 37° C., which it was felt would be safe for relatively long periods, and 47° C. which would give results more quickly but which might ultimately introduce undesirable high-temperature effects. The degree of acceleration produced by these temperatures could not accurately be predicted, since the temperature coefficient for deterioration of flavour in milk powder was not known. For the oxidation of fat the figure is probably of the order of 2·5 per 10° C.(7), and if this value be assumed also for the development of talliowiness in milk powder then the acceleration produced by storage at 37 and 47° C. as compared with a normal atmospheric temperature (taken as 15° C.) will be about 7·5 and 18·8 times, e.g. a period of 10 years at 15° C. will be reduced to 16 months at 37° C. or 6·4 months at 47° C. The majority of the results obtained to date on the absorption of oxygen, increase in peroxide value and development of talliowiness in milk powders at 37 and 47° C. have indicated temperature coefficients between 2·0 and 2·6, the average being about 2·2. The figures for talliowiness alone, which of course are only very approximate, indicate an average value of 2·15 between 47 and 37° C. and 2·05 between 37 and 15° C.

*Changes in oxygen concentration*

Figs. 2 and 3 give data for the disappearance of oxygen and production of carbon dioxide in sealed cans of milk powders containing different initial concentrations of oxygen. The percentages of oxygen and carbon dioxide recorded refer in each case to samples of gas withdrawn from the cans by the apparatus described elsewhere (Part IV). Obviously, the total pressure of gas in the can also diminishes as absorption proceeds by

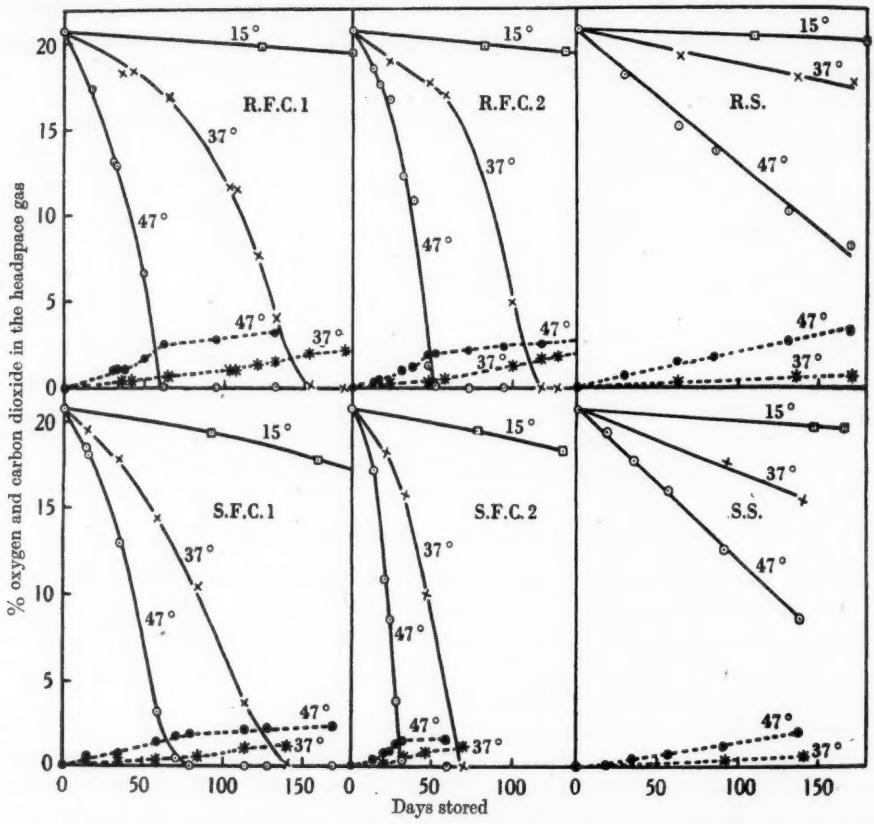


Fig. 2. Absorption of oxygen (—) and production of carbon dioxide (----) in cans of milk powder sealed in air and stored at 47, 37 and 15° C. R.F.C. roller full-cream; R.S. roller separated; S.F.C. spray full-cream; S.S. spray separated.

an amount corresponding to the difference between the absorption of oxygen and evolution of carbon dioxide. The percentages plotted in the figures can, if necessary, be converted to absolute amounts of oxygen or carbon dioxide per gram of powder by use of the weights of powder and volumes of free space given in Table 3, the temperature of sealing being in all cases about 20° C.

From these data it can be seen that the whole of the oxygen disappeared from air-packed cans of full-cream powder in from 1 to 2½ months at 47° C. and from 2 to 5 months at 37° C., appreciably sooner in fact than from some of the cans which commenced with

much smaller quantities of oxygen. Presumably the rapidly oxidizing fat in the air-cans displays a greater avidity for oxygen than the fat not yet completely out of its induction period in the gas packs. Comparison of Figs. 2 and 3 shows that reducing the pressure of oxygen in the can greatly slows down the rate of absorption of oxygen by the powder, and might therefore be expected to delay the appearance of defects associated with oxidation. The slower absorption of oxygen by separated powder was confirmed by experiment in glass apparatus at 60° C., whereby it was also shown that full-cream milk powder is able to absorb at least 15 times as much oxygen as is normally present in a can of the powder sealed in air, the experiment being discontinued at this point though absorption was still far from complete.

Appreciable differences were noticed in the rates at which individual powders of the same kind and from the same factory absorbed oxygen. In particular, the spray full-

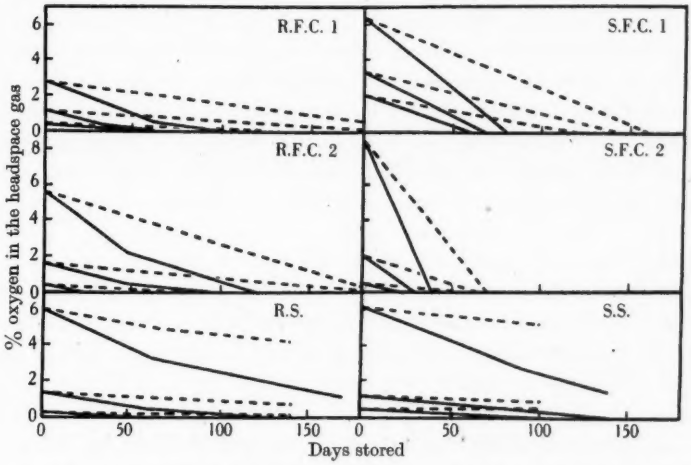


Fig. 3. Absorption of oxygen in cans of gas-packed milk powder at 47 (—) and 37° C (– – –).

cream no. 2 and roller full-cream no. 3 powders (Table 4), which were made from winter milks, were less stable than samples made in autumn, in spite of cooler weather conditions prevailing during transit and prior to packing.

### *Chemical changes in the fat*

*Extraction of the fat.* The amount of fat which can be extracted from spray-dried full-cream milk powders by direct treatment with solvents is only a small proportion (*ca.* 3–14 %) of that present, although in the case of the roller-dried powder extraction is almost complete (92–96 %). Lampitt & Bushill(8), however, have shown that if the moisture content of the powder is raised above a certain critical value (usually about 9 %) practically the whole of the fat becomes accessible to solvents, possibly as a result of the crystallization and disintegration of amorphous lactose which shields the fat in the dry powder from the solvent.

For extraction of the fat for examination the powders were therefore spread in thin layers in Petri dishes over water contained in shallow trays covered with glass plates, and held overnight at about 1° C. in the absence of light. Under these conditions the moisture

content of the powder increased to about 10 %. The fat was then extracted with peroxide-free ether (distilled from sodium) and the solvent distilled off, last traces being removed under high vacuum at a temperature not exceeding 60° C.

The iodine values of the fats extracted from the full-cream powders showed a progressive fall as the winter season advanced (Table 4), and would presumably have risen again to values of 44 or above with the return of the cows to fresh pasture in the spring.

The susceptibilities to oxidation of the fats extracted from the fresh powders were measured by heating at 65° C. under standard conditions with free access of air, and determining the peroxide values at intervals. The results (Table 4) show a slight tendency for the winter fats of lower iodine value to resist oxidation for longer periods, as recently found by Stebnitz & Sommer (9) for milk fats prepared by rendering fresh butter. On the other hand, in liquid milk the fat is generally considered to be *least* susceptible to oxidative taints when the cows are (or have recently been) receiving fresh pasture grass, possibly owing to the secretion in the milk serum of antioxidants derived from the grass. In the present case it was found that the two milk powders prepared in February were certainly the least stable of the group, in spite of the fact that their *extracted* fats had the lowest iodine values and were the most stable. These powders, however, also contained more copper than any others in the series, and it is obvious that a much larger group of samples prepared under more strictly standardized conditions would be necessary to establish a definite correlation between the resistance of milk powder to oxidative taints and the season.

*Determination of peroxide and Kreis values.* Peroxide values were determined on the extracted milk fats by the usual procedure (10), in which 1 g. of fat is heated with potassium iodide and 20 ml. of a mixture of glacial acetic acid and chloroform, and the iodine liberated titrated with 0·002 *N* thiosulphate solution. Larger quantities of fat (3–5 g.) were used when the titration expected was below 1 ml./g., and in cases where the peroxide value exceeded 10 ml./g. the weight of fat taken was reduced until the product of the weight and the actual titration obtained was of the order of 10 (e.g. 0·7 g. when the peroxide value was 20 ml./g.). In this way the sensitivity of the method was increased in its lowest ranges, and the loss by reabsorption of iodine minimized when the peroxide value was high.*

The Kreis test was carried out according to the technique of Walters, Muers & Anderson (11), using an amyl acetate solution of phloroglucinol with trichloracetic acid, in place of the usual ethereal or alcoholic solution of phloroglucinol and hydrochloric acid. Some difficulty was experienced in obtaining reproducible results when the degree of dilution and the size of the cell used were varied, and it was found more satisfactory to standardize the degree of dilution at 10 ml. and the size of cell at 0·5 cm. Under these conditions Kreis values up to 40 could be determined, and this range covered all the samples examined. Reproducibility at the lower end of the scale was usually of the order of 1 or 2 units, but a change of observer at the tintometer could introduce a greater difference.

Peroxide and Kreis values determined on fats extracted from the various milk powders after storage at 47 and 37° C. are given in Table 5, the data for the air-stored samples being given in greater detail in Fig. 4. A striking point is the dependence of both tests on

* Precautions against loss of iodine by reabsorption become increasingly important with increasing iodine value of the fat under investigation. In the case of butter fat there is little loss of accuracy in taking 1 g. of fat even for values above 20 ml./g., and for most purposes a standard 1 g. sample is quite satisfactory.

Table 4. *Properties of the milk powders used*

| | Roller full-cream | Spray full-cream | Spray | Roller |
|---|---|---|---|---|

Table 4. *Properties of the milk powders used*

| | Roller full-cream | | | Spray full-cream | | Roller separated | Spray separated |
|---|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 1 | No. 2 | | |
| Date of manufacture | 29 Sept. | 23 Nov. | 21 Feb. | 1 Nov. | 7 Feb. | 27 Oct. | 3 Jan. |
| Fat content (%) | 26·8 | — | — | 27·2 | — | 1·0 | 2·4 |
| Moisture content (%) | 2·9 | 2·4 | 3·7 | 1·3 | 1·3 | 4·0 | 1·3 |
| Copper content (p.p.m.) | 2·3 | 4·8 | 5·0 | 2·1 | 9·2 | 3·1 | 1·5 |
| *Days to acquire a tallowy flavour of strength 2 in air-pack at: 47° C. | 20 | 18 | 15 | 16 | 14 | *ca.* 35† | *ca.* 60† |
| 37° C. | 40 | 40 | 30 | 35 | 32 | — | — |
| 15° C. | 230 | 180 | — | 170 | 130 | — | — |
| Iodine value of the fat | 44·2 | 40·8 | 38·5 | 43·1 | 38·9 | — | — |
| Induction period of the extracted fat‡ | 200 | 190 | 230 | 180 | 380 | — | — |

* Details recorded in Part II. † General palatability. ‡ Hours at 65° C. to reach a peroxide value of 5 ml./g.

Table 5. *Changes in peroxide and Kreis values in gas-packed milk powders*

| Initial concentration of $O_2$ % | Initial values | | Days at 47° C. | | | | | | | | Days at 37° C. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P. | K. | P. | K. | P. | K. | P. | K. | P. | K. | P. | K. | P. | K. | P. | K. |
| | | | Roller full-cream no. 2 | | | | | | | | | | | | | |
| | | | 23 | | 47 | | 95 | | 156 | | 199 | | 58 | | 118 | | 186 | |
| 20·8 | 0·2 | 9 | 1·6 | 14 | 18·6 | 28 | 8·6 | 16 | 6·3 | 12 | 3·9 | 14 | 2·2 | 19 | 24·9 | 17 | 14·0 | 16 |
| 5·6 | 0·2 | 9 | 0·4 | 9 | 0·5 | 10 | 0·6 | 9 | 0·9 | 10 | 0·5 | 10 | 0·6 | 12 | 1·2 | 12 | 3·4 | 13 |
| 1·6 | 0·2 | 9 | 0·3 | 8 | 0·3 | 10 | 0·3 | 12 | 0·3 | 10 | 0·3 | 11 | 0·4 | 12 | 0·3 | 9 | 0·7 | 9 |
| 0·4 | 0·2 | 9 | 0·2 | 6 | 0·2 | 10 | 0·2 | 9 | 0·3 | 10 | 0·2 | 10 | 0·3 | 11 | 0·2 | 10 | 0·2 | 9 |
| | | | Spray full-cream no. 1 | | | | | | | | | | | | | |
| | | | 80 | | 128 | | 169 | | 196 | | | | 85 | | 140 | | 206 | |
| 20·8 | 0·3 | 9 | 4·7 | 13 | 3·3 | 10 | 1·5 | 11 | 1·0 | 10 | — | — | 5·7 | 16 | 5·5 | 14 | 4·4 | 14 |
| 6·4 | 0·3 | 9 | 1·8 | 11 | 1·3 | 10 | 0·5 | 8 | 0·4 | 10 | — | — | 2·0 | 14 | 1·8 | 10 | 1·6 | 12 |
| 3·3 | 0·3 | 9 | 1·1 | 11 | 0·5 | 8 | 0·3 | 10 | 0·2 | 10 | — | — | 1·0 | 10 | 1·2 | 9 | 0·9 | 9 |
| 1·9 | 0·3 | 9 | 0·6 | 8 | 0·5 | 10 | 0·2 | 5 | 0·1 | 8 | — | — | 0·8 | 8 | 0·9 | 9 | 0·4 | 8 |
| | | | Spray full-cream no. 2 | | | | | | | | | | | | | |
| | | | 32 | | 59 | | 97 | | | | | | 47 | | 70 | | 110 | |
| 20·8 | 0·4 | 4 | 9·4 | 27 | 7·0 | 20 | 4·6 | 13 | — | — | — | — | 8·4 | 22 | 11·6 | 21 | 8·3 | 19 |
| 8·4 | 0·4 | 4 | 3·1 | 14 | 3·2 | 11 | 2·0 | 10 | — | — | — | — | 3·4 | 12 | 5·3 | 15 | 3·4 | 16 |
| 2·1 | 0·4 | 4 | 1·2 | 8 | 0·5 | 7 | 0·4 | 7 | — | — | — | — | — | — | — | — | — | — |
| 0·6 | 0·4 | 4 | 0·7 | 6 | 0·3 | 6 | 0·2 | 5 | — | — | — | — | — | — | — | — | — | — |
| 0·2 | 0·4 | 4 | 0·3 | | 0·2 | 6 | 0·1 | 4 | — | — | — | — | — | — | — | — | — | — |

the presence of free oxygen in the containers. Both reach a maximum just before all the oxygen has been consumed and then fall more or less rapidly. Higher values were reached in the roller than in the spray powders, probably owing to the considerably greater volume of air per gram of powder present in cans of the former. The increases in peroxide and Kreis values in the gas-stored samples were small, particularly in case of the roller powders.

Obviously the compounds responsible for the Kreis reaction, as well as the fat peroxides, are intermediate products in the oxidation of fat and only accumulate in the presence of

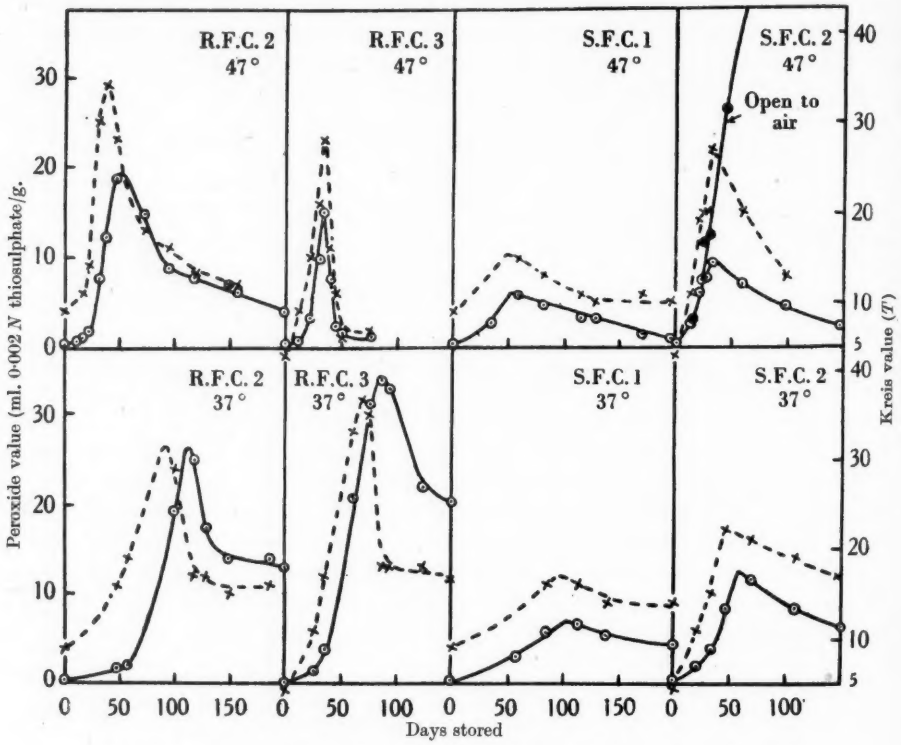


Fig. 4. Changes in peroxide (⊙) and Kreis (×) values of the fat of milk powders sealed in air and stored at 47 and 37° C.

free oxygen by the preponderating effect of the reactions forming them over those decomposing them. The fact that an appreciably higher maximal value for the peroxide test is recorded at 37 than at 47° C. suggests that the temperature coefficient of the decomposing reaction is greater than that of the formative reaction, and that higher maximal values would be reached during storage at atmospheric temperature. The few figures obtained from powders stored in air at 15° C. are given in Fig. 5. Two old samples of roller full-cream powder which had been sealed in air and stored for 10–15 years at room temperature gave peroxide values of 10–13 ml./g. of fat.

The extremely rapid fall in both peroxide and Kreis values recorded in case of roller powder no. 3 at 47° C. is abnormal, since this powder darkened very rapidly at 47° C.,

with charring and the production of large amounts of carbon dioxide. Even with free exposure to the air the peroxide and Kreis values fell away to very low values as charring developed. Roller powder no. 1 behaved similarly to no. 2.

The fact that the concentration of peroxide commences to fall as soon as the free oxygen has disappeared from the can is to be expected from the generally accepted theory of the oxidation of fat, according to which molecular oxygen is added on to the double bonds of the unsaturated glycerides to form peroxides which subsequently isomerize, break down or are further oxidized to a variety of secondary oxidation products including aldehydes, acids and keto-hydroxy compounds. The precise route by which the epihydrin aldehyde, which is considered to be the product responsible for the Kreis test, is formed from the

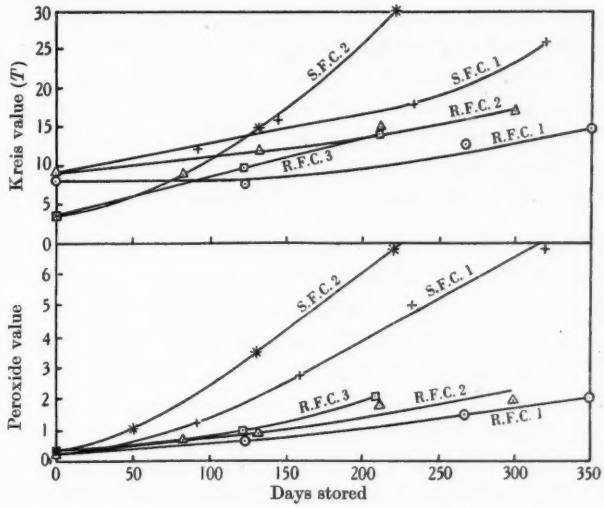


Fig. 5. Chemical changes in the fat of milk powders in air at 15° C.

peroxide is obscure. In the present experiments the Kreis value commenced to fall as soon as, or even earlier than, the peroxide value. The Kreis test therefore does not appear to possess any advantage over the peroxide test for following the oxidation of fat in a restricted supply of oxygen. Low values for both tests can obviously be obtained from tallowy powders after very prolonged storage in sealed containers.

## SUMMARY OF PART I

Experiments in which butter fat was heated in sealed tubes with known amounts of oxygen indicated the order of magnitude of the maximum concentration of oxygen permissible in the head-space gas of cans of milk powder if tallowy flavours due to oxidation of the fat were to be prevented.

Chemical tests on fat extracted from the powders showed that with the disappearance of free oxygen from the container both the peroxide and Kreis values passed through a maximum and fell away again to low values.

## Part II. CHANGES IN PALATABILITY

C. H. LEA and J. A. B. SMITH

### Assessment of flavour

From a purely practical point of view the most important changes occurring in milk powder on storage are changes in odour and flavour. The quantitative assessment of flavour is always a matter of difficulty owing to the variation in sensitivity found between different individuals and for the same individual at different times, to the fatigue and resulting loss in sensitivity experienced in tasting a series of samples, and to the disturbing influence of the other senses and of psychological factors, which may in themselves be sufficiently strong to invalidate completely the judgment given. When, as in the present case, a series of observations over a period of months is required further difficulty is experienced in maintaining a reasonably consistent standard of grading. For this reason, and because it was desired to average the marks given by a tasting panel of five people, a scale of only five grades was adopted, each representing a condition easily describable in words. The grades were defined as follows:

0 = as good as the control. 2 = a definite 'off' flavour. 4 = very unpalatable.
1 = a suspicion of 'off' flavour. 3 = unpalatable.

Samples averaging below 1 would be considered good, between 1 and 2 usable, and between 2 and 3 unusable (except perhaps for cooking or manufacture). Samples averaging above 3 were very unpleasant to taste. There is, of course, no suggestion of proportionality between the number given according to this scale and the intensity of 'off' flavour; in a sample marked '3' or '4', for example, the 'off' flavour would be many times stronger than in a sample marked '1'. An approximately straight line flavour/time relationship from zero to 3 or 4 as frequently obtained does not therefore indicate a uniform increase in intensity of flavour with time, but is consistent with the existence of an appreciable induction period as shown by the oxygen absorption and peroxide curves. The scale used has, however, the advantage of sensitivity in the earlier and more important stages of deterioration. Later on, when, for example, it becomes necessary to decide whether a sample is 'unpalatable' or 'very unpalatable', some inconsistency is inevitable, though this is reduced by averaging the verdicts of five independent tasters, and by having tasting panels at three different research laboratories. In a few instances tests were also made by groups of untrained observers.

For tasting, the cans were opened and the powders immediately mixed with water at 60° C. in the proportion of 14 g. (full-cream powder) or 10 g. (separated powder) to 100 ml. of water, stirred well and cooled to 20° C., each sample being distinguished only by a key number allotted to it by a person not a member of the panel. The tasters were then asked to place the samples in order of preference, grouping together any which could not be distinguished and, in addition, to award marks in accordance with the scale for (*a*) 'general palatability' and (*b*) 'tallowiness'. The average order of preference and the average mark under each category were then calculated for the panel.

This system of examining the reconstituted milk rather than the powder itself was adopted after preliminary trials because it was found to yield more consistent results.

The odour of the powder after standing in a stoppered bottle certainly offers a very sensitive indication of deterioration, but is troublesome to apply in a standard way as a routine test. Tasting the solid powder is of relatively little use because the sweet taste due to a local concentration of lactose on the tongue tends to mask 'off' flavour, while at the same time the solid powder clogs the palate and induces rapid fatigue. Even in tasting reconstituted milks it was found to be advisable to place the samples roughly in order according to odour, and then to taste them in that order, starting with the best. Members of the regular panel could usually recognize the 'hidden' control as the sample with the least 'heated' flavour, and a majority of them preferred it for this reason (Table 6). A minority on the panel at Cambridge and frequently a majority of untrained observers, however, consistently preferred the rather fuller and richer flavour of the best of the gas-stored samples. A similar variation in personal preference was found in the tasting tests at the laboratories of both the Hannah Institute and Messrs Lyons.

### Types of flavour

*Tallowiness.* In air the full-cream powders showed a suspicion of tallowiness after about 10 days at 47° C., definite tallowiness after about 15 days and were classed as unpalatable after about 20–30 days. After 40–60 days all the free oxygen had disappeared from the cans, and no further marked change in *degree* of unpalatability was detected, although, as in the case of butter fat (Part I), the *character* of the off flavour changed appreciably on continued heating and became less characteristically 'tallowy'.

Reduction of the concentration of oxygen in the free space in the cans to 3–6 % kept the powder free from tallowiness for a period of the order of three times as long as in air, and the maximum intensity of the 'off' flavour subsequently attained was much less (Fig. 6).

Further reduction of the concentration of oxygen to below 1 % in case of roller full-cream powder or 2 % in case of the spray powder (corresponding in each case to about 0·02 ml. of oxygen per g. of powder, or 0·08 ml./g. of fat) produced a further improvement in keeping properties, and tallowiness could not be definitely identified (i.e. the 'tallowy' flavour failed to exceed '1') in powders stored under these conditions. In one or two cases, however, powders packed in concentrations of oxygen only slightly exceeding these limits approached fairly closely to flavour '1', and for this reason a slightly lower limit (e.g. 0·5 % for roller, 1·0 % for spray powder) is probably desirable. Marked 'tallowy' flavours were not observed below about 3–5 % of oxygen.

Results at 37° C. generally confirmed those at 47° C. (Fig. 6).

*'Burnt', 'Toffee' and 'Stale' flavours.* Powders which had been stored for some time at 47 and 37° C. could be distinguished from fresh controls by a flavour variously described as slightly 'heated', 'burnt', 'scorched' or 'cooked', which probably consisted of two components, namely, (*a*) a 'burnt' or 'caramel' taste associated with the protein or carbohydrate, and (*b*) a 'butter-toffee' flavour associated with the fat.

Fresh milk powders, particularly roller powders, already taste slightly 'burnt' by comparison with fresh milk, and a slight increase in this flavour does not seriously impair palatability. The burnt taste did not seem to increase progressively on storage, and was less noticeable in the spray than in the roller powders. Packing in low concentrations of oxygen appeared to reduce the intensity of the flavour, but failed to prevent it.

'Toffee' flavour, which had already been encountered in the experiments on butter fat

Table 6. *Effect of gas storage on palatability (order of preference)*

| | Initial concentration of $O_2$ % | Days at 47° C. | | | | | | | | Days at 37° C. | | | | | Average order of preference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Roller full-cream no. 1 | Fresh control | 33* | 62* | 95* | 132* | 204† | | | | 65* | 107* | 121‡ | 175† | | — |
| | 0·5 | 1·2 | 1·6 | 1·2 | 2·2 | 1·0 | | | | 1·3 | 1·4 | 1·8 | 1·4 | | 1·5 |
| | 1·1 | 2·6 | 2·4 | 2·0 | 1·7 | 2·5 | | | | 2·6 | 1·5 | 2·2 | 1·9 | | 2·2 |
| | 2·8 | 2·6 | 2·1 | 3·0 | 2·1 | 2·5 | | | | 2·8 | 2·8 | 2·0 | 2·8 | | 2·5 |
| | 20·8 | 3·5 | 3·9 | 3·9 | 4·0 | 4·0 | | | | 3·4 | 4·0 | 4·0 | 3·9 | | 3·8 |
| | | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 | | | | 5·0 | 5·0 | 5·0 | 5·0 | | 5·0 |
| | | 23* | 47* | 95† | 157‡ | 199* | | | | 23‡ | 58† | 118‡ | 128† | 186‡ | |
| Roller full-cream no. 2 | Fresh control | 1·6 | 1·6 | 1·7 | 1·0 | 1·1 | | | | 1·8 | 1·3 | 1·3 | 1·1 | 1·0 | 1·4 |
| | 0·4 | 2·2 | 1·9 | 1·9 | 2·2 | 2·0 | | | | 2·8 | 2·1 | 1·7 | 2·1 | 2·1 | 2·1 |
| | 1·6 | 3·0 | 2·6 | 2·6 | 3·2 | 3·0 | | | | 2·4 | 3·3 | 3·2 | 2·8 | 2·9 | 2·9 |
| | 5·6 | 3·7 | 3·9 | 3·8 | 3·8 | 4·0 | | | | 3·2 | 3·4 | 3·8 | 4·0 | 4·0 | 3·8 |
| | 20·8 | 4·6 | 5·0 | 5·0 | 4·8 | 5·0 | | | | 4·8 | 5·0 | 5·0 | 4·9 | 5·0 | 4·9 |
| | | 15‡ | 35* | 59* | 80‡ | 128† | 169† | 177‡ | 196* | 35‡ | 59* | 85‡ | 140‡ | 206‡ | |
| Spray full-cream no. 1 | Fresh control | 1·4 | 1·2 | 1·3 | 1·8 | 1·7 | 1·2 | 1·0 | 1·0 | 1·4 | 1·8 | 1·6 | 1·4 | 1·0 | 1·4 |
| | 1·9 | 2·5 | 2·4 | 2·6 | 2·4 | 2·0 | 2·5 | 2·2 | 2·1 | 1·6 | 2·1 | 1·7 | 1·6 | 2·0 | 2·1 |
| | 3·3 | 3·2 | 3·0 | 2·5 | 2·6 | 2·5 | 2·4 | 2·8 | 2·9 | 3·4 | 2·8 | 3·0 | 3·0 | 3·0 | 2·9 |
| | 6·4 | 2·9 | 3·9 | 3·8 | 4·0 | 3·8 | 3·9 | 4·0 | 4·0 | 3·6 | 3·5 | 3·7 | 4·0 | 4·1 | 3·8 |
| | 20·8 | 5·0 | 4·6 | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 | 4·9 | 5·0 |
| | | 32‡ | 59‡ | 97* | | | | | | 70‡ | 110‡ | | | | |
| Spray full-cream no. 2 | Fresh control | 2·1 | 1·1 | 1·3 | | | | | | 2·1 | 1·9 | | | | 1·7 |
| | 0·2 | 2·7 | 2·0 | 2·3 | | | | | | 2·2 | 2·7 | | | | 2·4 |
| | 0·6 | 2·3 | 3·0 | 2·7 | | | | | | 2·4 | 2·6 | | | | 2·6 |
| | 2·1 | 2·9 | 3·9 | 3·6 | | | | | | 3·3 | 2·7 | | | | 3·3 |
| | 8·4 | 5·0 | 5·0 | 5·0 | | | | | | 5·0 | 5·0 | | | | 5·0 |
| | 20·8 | 6·0 | 6·0 | 6·0 | | | | | | 6·0 | 6·0 | | | | 6·0 |
| | | 29* | 62* | 131† | 170‡ | | | | | 63* | 136‡ | | | | |
| Roller separated | Fresh control | 2·9 | 2·1 | 2·3 | 1·7 | | | | | 2·3 | 3·9 | | | | 2·5 |
| | 0·2 | 2·5 | 2·8 | 1·9 | 1·9 | | | | | 2·7 | 4·5 | | | | 2·7 |
| | 0·3 | 3·0 | 2·3 | 2·8 | 3·5 | | | | | 3·0 | 3·0 | | | | 2·9 |
| | 1·4 | 3·7 | 3·3 | 3·1 | 3·6 | | | | | 3·3 | 2·2 | | | | 3·2 |
| | 6·0 | 4·3 | 4·6 | 5·2 | 5·0 | | | | | 4·5 | 1·8 | | | | 4·2 |
| | 20·8 | 4·8 | 5·5 | 5·8 | 4·8 | | | | | 5·2 | 5·6 | | | | 5·3 |
| | | 35* | 59‡ | 91‡ | 138‡ | | | | | 93‡ | | | | | |
| Spray separated | Fresh control | 1·5 | 1·3 | 2·4 | 2·2 | | | | | 2·0 | | | | | 1·9 |
| | 0·5 | 2·8 | 2·7 | 2·6 | 2·2 | | | | | 2·0 | | | | | 2·5 |
| | 1·1 | 3·1 | 2·5 | 2·6 | 2·5 | | | | | 2·5 | | | | | 2·6 |
| | 6·2 | 2·9 | 3·7 | 2·4 | 3·0 | | | | | 3·5 | | | | | 3·1 |
| | 20·8 | 4·7 | 3·8 | 5·0 | 5·0 | | | | | 5·0 | | | | | 4·7 |

* Average of results by panels at L.T.R.S. and either Lyons' laboratories or the Hannah laboratories.
† Average of results from all three panels. ‡ L.T.R.S. panel only.

(Part
compl
this fl
and t
5–7 m
usable

Tallowy flavour

A
prot
47°
was
afte
thos
Qua
diff

(Part I), developed in both gas-packed roller and spray full-cream powders, but was completely masked by tallowiness in air. Up to about 4–6 months at 47° C. the effect of this flavour was usually to increase the apparent 'richness' or 'creaminess' of the milk, and the reconstituted fluid was quite palatable. In some cases, however, the flavour after 5–7 months was too strong to be pleasant in liquid milk, though the powder would still be usable for cooking or manufacturing purposes.

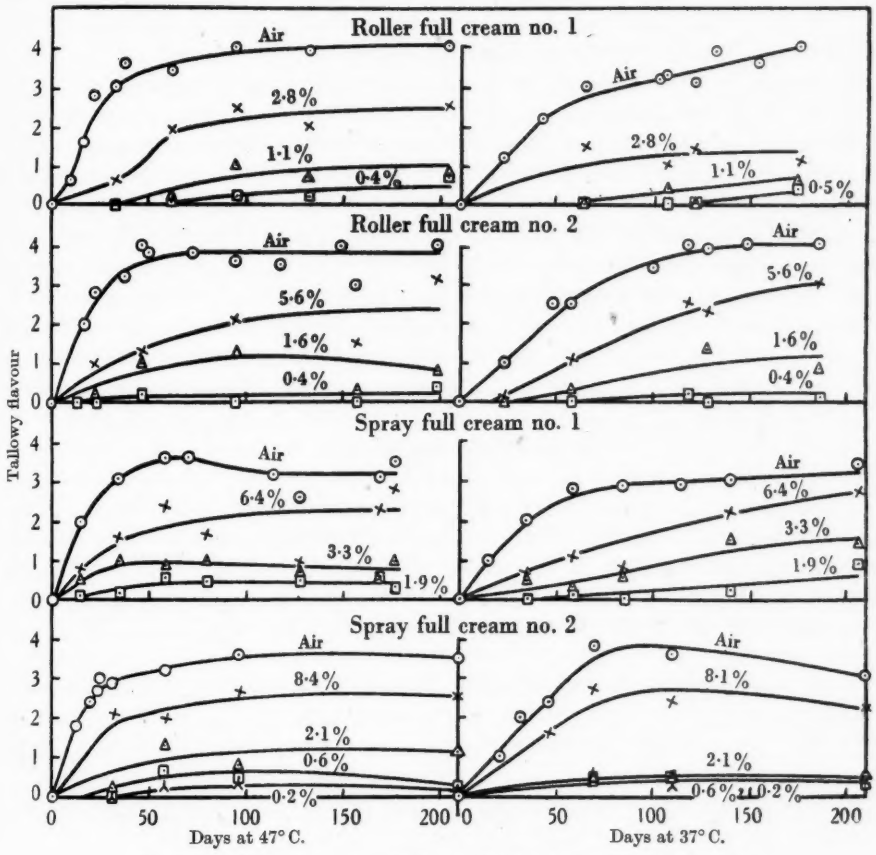


Fig. 6. Effect of oxygen concentration on the development of tallowiness in full-cream milk powder (L.T.R.S. panel).

A slight 'off' flavour described as 'stale' and of a type likely to be associated with protein decomposition appeared in the gas-stored roller powders after 4–5 months at 47° C., but had not after 6 months been identified in the spray-dried powders. The flavour was detectable also in the roller full-cream powders at the lower temperatures, in fact after storage for 5 or 6 months the powders at 37 and 47° C. were sometimes preferred to those at −20° C., owing to the effect of the slight 'heated' taste in masking the stale flavour. Quantitative assessment of slight 'off' flavours of this kind against a fresh control of a different sample of powder, however, is difficult, and further investigation of stale flavour

and of the effect of temperature on its production is obviously necessary. In any case it must be emphasized that abnormal flavours developed by storing gas-packed full-cream powders for 5 or 6 months at 47° C., or considerably longer at 37° C., were much less serious in their effect on palatability than the 'tallowy' flavours developed in about 3 weeks at 47° C. by the powders in air.

*Separated powders.* The only roller-separated powder stored contained 1·0 % of fat and 4·0 % of water,* and was not palatable even when fresh owing to the presence of a decidedly 'burnt' and 'stale' or 'glue-like' flavour. On keeping in air no obvious tallowiness developed, but the 'off' flavour gradually became more pronounced.

The gas-packed powders deteriorated rather less rapidly, but the correlation between degree of 'off' flavour and initial oxygen content of the can was much less clear than with the full-cream powders. In air, the roller-separated powder appeared to keep without serious deterioration in flavour for about twice as long as the corresponding full-cream powder.

A spray-dried separated powder (2·4 % fat, 1·4 % moisture) had only a suspicion of 'stale' flavour when fresh and was perfectly palatable. It developed tallowiness on storage in air, though much less rapidly than the full-cream powders and, in fact, appeared to keep in a palatable condition for about three times as long as the latter. The gas-stored samples after 5–6 months at 47° C. had developed a slight 'heated' taste, but were not 'tallowy' and there had been no appreciable increase in stale flavour; the powders were still quite palatable.

Differences in palatability between fresh controls and samples of milk powder stored at 47° C. for several months in low concentrations of oxygen were not very pronounced. This is demonstrated by the chaotic results obtained when twenty people unused to the critical tasting of milk powders were asked to place four samples of a full-cream powder in order of preference (Table 7). The powder used for this test had rather poor keeping properties, and when stored in air was definitely inferior to the control, owing to talloviness, after 10–14 days at 47° C.

Table 7. *Palatability of spray full-cream powder after storage at* 47° C. *for* 100 *days*

| Initial concentration of $O_2$ | L.T.R.S. panel, 5 tasters | | | | | Hannah panel, 4 tasters | | | | Average order of preference |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 2·5 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1·3 |
| 0·2 | 2·5 | 2·5 | 2 | 2 | 1 | 3 | 3 | 3 | 2 | 2·3 |
| 0·6 | 2·5 | 2·5 | 3 | 3·5 | 3 | 2 | 2 | 2 | 4 | 2·7 |
| 2·1 | 2·5 | 4 | 4 | 3·5 | 4 | 4 | 4 | 4 | 3 | 3·7 |
| 8·4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5·0 |
| 20·8 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6·0 |

| | Untrained observers, 20 tasters | | | | | | | | | | | | | | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 1 | 1 | 2 | 1 | 1 | 4 | 3 | 3 | 1 | 4 | 3 | 4 | 1 | 2 | 2 | 1 | 2·5 | 1 | 1·5 | 3·5 | 2·1 |
| 0·2 | 4 | 2 | 1 | 4 | 3 | 1 | 1 | 1 | 2 | 3 | 2 | 2 | 3 | 2 | 1 | 2 | 2·5 | 2 | 1·5 | 3·5 | 2·2 |
| 0·6 | 3 | 3 | 3 | 2 | 4 | 2 | 2 | 4 | 3 | 2 | 1 | 1 | 2 | 2 | 3 | 3·5 | 4 | 4 | 3·5 | 1·5 | 2·7 |
| 2·1 | 2 | 4 | 4 | 3 | 2 | 3 | 4 | 2 | 4 | 1 | 4 | 3 | 4 | 4 | 4 | 3·5 | 1 | 3 | 3·5 | 1·5 | 3·0 |

## Discussion

The experiments on butter fat and on milk powders have indicated that the production of a strong 'tallowy' odour and flavour, which is the main cause of spoilage in full-cream milk powders stored in air, can be prevented by packing the powder in gas-tight containers

* Equivalent to approximately 3 % in a full-cream powder.

in an atmosphere of nitrogen containing not more than about 0·5–2 % of oxygen. Since the powders were stored until all the free oxygen in the container had been absorbed, and most of the reactive peroxide first formed destroyed, it seems reasonable to assume that under these conditions tallowiness would be prevented for an indefinite period. The experiments on both butter fat and milk powder suffered from the disadvantage that storage at the high temperatures necessary for conclusion of the experiments in a reasonable length of time produced other flavour changes which might have masked very slight 'off' flavours due to oxidation of the fat. There is no doubt, however, of the beneficial effect of packing in inert gas, and that the harmful influence of fat oxidation on flavour can be reduced to very small proportions, if not completely eliminated in this way.

With the suppression of tallowiness, normally the principal factor in the deterioration of full-cream powders, other less marked flavour changes appeared. The slight 'heated' or 'burnt' flavour characteristic of certain milk products increased, though not progressively, when the powders were stored at 47 and, to a smaller extent, 37° C., and helped to distinguish gas-stored powders from fresh controls. 'Burnt' flavour seemed to decrease with decreasing concentration of oxygen, thereby enabling the panel to place the gas-stored samples in correct order even at oxygen concentrations below those necessary for identifiable tallowiness. The cause of these flavours is not known, but is presumably associated with changes in the protein or carbohydrate fractions of the milk. 'Burnt' or 'caramel' tastes are known to be produced in liquid milk by brief exposure to ultraviolet light (12), and by storage under a pressure of 8 atm. of oxygen at 8° C. for 3 or 4 weeks (13). It seems, therefore, that production of this flavour is not exclusively a high-temperature phenomenon, and that the flavour may perhaps increase in gas-packed milk powders on prolonged storage at ordinary temperature.

The 'butter-toffee' flavour already referred to is apparently due to changes in the flavouring constituents of the fat, changes which may be independent of oxygen since they were not prevented by the lowest concentration of oxygen achieved in the experiments. No mention of this flavour appears to have been made in the literature on the storage of milk powder, and it is not clear whether the production of a toffee-like flavour is purely a result of the high temperatures employed in the accelerated technique, or whether it will ultimately develop in full-cream powders stored in inert gas at ordinary temperature. In the latter case the defect is hardly likely to assume serious proportions until the powder has been stored for several years.

Slight burnt and toffee-like flavours, such as were observed in the present experiments after storage in 'nitrogen' for 4–6 months at 47° C.* did not seriously impair palatability, though they rendered the reconstituted fluids rather less like fresh milk. A majority of the members of the tasting panels definitely preferred the samples with the least heated flavour, though it was impossible to form a really unbiased opinion since the fresh control could be recognized. As already indicated, differences in palatability between the fresh control and samples stored at 47° C. for several months in low concentrations of oxygen were not very pronounced (Table 7).

The flavours described in this paper as 'burnt' and 'stale' obviously have something in common, and it is possible that the less unpleasant 'burnt' flavour is in fact a compound produced by the imposition of a toffee flavour in the fat and perhaps slight caramelization of the lactose on to the 'stale' flavour resulting from changes in the protein. 'Stale'

* Equivalent to 6–9 years at 15° C., assuming a temperature coefficient of 2·5.

flavours developed by the roller full-cream powders were never strong, and it is by no means certain that they will increase progressively on further storage. Also, it may be possible to prevent them by such means as further reducing the moisture content of the powder. It is significant that the spray-dried powders, which had been least heated during manufacture and possessed the lowest moisture contents, had not in 6 months at 47° C. shown any definite production of the flavour; nor had the gas-packed powders after 12 months at 15° C. developed any sign of 'off' flavour of any description. The fact, however, that 'stale' flavours were detected at all in some of the roller-dried powders after a few months at 15 and even at −20° C., indicates that for this form of spoilage the accelerated test at high temperatures may break down as a means of estimating keeping properties over long periods. Further investigation of the nature of 'stale' flavour and of the influence of moisture content and degree of heating of the powder during preparation, as well as of the conditions of packing and temperature of storage will therefore be necessary before the extent to which the storage life of milk powders can be prolonged by gas packing can finally be elucidated.

SUMMARY OF PART II

Accelerated tests in which full-cream milk powders were stored in nitrogen containing various concentrations of oxygen at 47 and 37° C. confirmed the findings for butter fat, and indicated that inert gas containing not more than 0·5–1 % of oxygen (i.e. 0·01 ml. of oxygen per g. of powder) should prevent the development of recognizable 'tallowy' odours and flavours for an indefinite period, while nitrogen containing as much as 3–6 % of oxygen should still have a very marked effect in retarding the development of tallowiness.

## PART III. THE PREVENTION OF LOSS OF SOLUBILITY

C. H. LEA AND J. A. B. SMITH

### INTRODUCTION

Freshly prepared milk powder varies considerably in 'solubility', i.e. in the extent to which it is capable when mixed with the appropriate quantity of water of simulating the physical characteristics of natural milk. Exposure to high temperatures during pre-condensing or drying reduces the solubility of the powder. In roller drying at atmospheric pressure, where evaporation of the film of milk in contact with the heated metallic surface is comparatively slow, solubility is impaired and atmospheric roller powders are usually only soluble to the extent of about 70–85 % in cold water or 80–95 % in hot. In spray drying, on the other hand, the extremely rapid evaporation maintains at first the temperature of the particles at a very low figure, and minimizes that exposure of the concentrated but not fully dried milk to high temperature which has been shown to be most destructive of solubility (14). Spray-process powders are therefore practically completely soluble in cold water. Roller drying in excessively thin films at low temperatures in air, or roller drying *in vacuo* is said to yield a product approaching the spray-dried material in solubility (15, 16), but no such roller-process milk appears to be produced in this country. Milk that is not fresh and has developed acidity or coagulating enzymes due to bacterial action is liable to yield a powder of diminished solubility.

The solubility of milk powder tends to diminish with age, the change increasing rapidly with increasing moisture content and rise in storage temperature. Apparently the decrease in solubility is due to physico-chemical changes in the protein (chiefly in the casein) which

occur much more rapidly in moist than in dry powders. If the moisture content of the powder is kept below 3 %, the original solubility is said to be maintained practically unchanged for a year(17). The solubility of the casein in a sample of roller-dried milk powder of which the moisture content was maintained between 2·3 and 3·5 % was found to decrease only from 77·3 to 71·6 % in 329 days. On the other hand, in a sample of powder maintained at 5 % moisture content for 273 days the solubility decreased from 77·3 to 6·5 %(18).

No data, however, appear to be available concerning changes in the solubility of powders of relatively low moisture content over very long periods. Since it was not practicable to wait several years for results, use had to be made of accelerated tests, and an investigation has therefore been made of the changes in solubility undergone by powders stored in sealed containers at 37 and 47° C.

Accurate information concerning the acceleration of the production of insolubility in milk powders of normal moisture content by heating at temperatures between 15 and 47° C. is not available, but Wright(14) has published data for separated milks containing from 13·3 to 92 % of water which show that the temperature coefficient of the reaction over this range is very high, in fact about 4·46. Since the temperature coefficient for a powder containing 2·6 % of moisture over the range 100–140° C. was even higher (6·32), it seems reasonable to suppose that the value for the powders used in the present work (1·3–4·0 % moisture content) over the range 15–47° C. would not be less than 4.

## Experimental

Milk powders of various types, i.e. roller full-cream, roller separated, spray full-cream and spray separated were packed in air and in nitrogen containing various known concentrations of oxygen and stored at 37 and 47 ± 0·1° C. for periods up to 6 or 7 months. At intervals cans were opened and solubility determined both at 20 and at 50° C.

The samples referred to as 'controls' (Table 8) were portions of the original powder which had been stored in sealed containers at −20° C. under the lowest concentration of oxygen available in the particular series. These samples would not be expected to change appreciably in solubility during the course of the experiment, and therefore serve as a useful check on the reproducibility of the method. Comparison of the values obtained from the samples stored at 47 or 37° C. with that of a 'control' determined at the same time probably gives a more accurate picture of the changes occurring in the heated powders than would direct comparison with the values obtained on the fresh powder at the beginning of the experiment.

In principle the method employed for the measurement of solubility consists in shaking or stirring the powder with the requisite quantity of water at the desired temperature, centrifuging and determining the amount of dry material present in an aliquot portion of the supernatant fluid. In practice, the conditions employed must be very carefully controlled to obtain reproducible results, and the values given by slightly different techniques vary considerably.

In all tests made at the Hannah Institute, the process used for the determination of solubility was the rapid method described by Howat, Smith, Waite & Wright(19). In this method the soluble solids of the milk are estimated in such a way that the lactose, originally present almost entirely in the anhydrous form, crystallises with one molecule of water. A small correction to allow for this has therefore been applied in arriving at the values recorded in the present paper.

Table 8. *Changes in solubility of milk powders during storage at 47 and 37° C.*

*Roller full-cream no. 1, 2·9 % moisture*

| Initial concentration of oxygen % | Days at 47° C. | | | | | Days at 37° C. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 33 | 62 | 95 | 204 | 0 | 65 | 107 | 175 |
| | Solubility at 20° C. (Hannah) | | | | | | | | |
| 20·8 | 75 | 73 | 71 | 77 | 72 | 75 | 80 | 72 | 78 |
| 2·8 | 75 | 72 | 74 | 73 | 74 | 75 | 76 | 75 | 78 |
| 1·1 | 75 | 73 | 71 | 73 | 74 | 75 | 74 | 72 | 78 |
| 0·5 | 75 | 75 | 74 | 75 | 76 | 75 | 74 | 79 | 78 |
| Control | 75 | 75 | 77 | 77 | 79 | 75 | 78 | 78 | 82 |
| | Solubility at 20° C. (Lyons) | | | | | | | | |
| 20·8 | 71 | 71 | 68 | 72 | | 71 | 74 | | |
| 2·8 | 71 | 68 | 63 | 68 | | 71 | 72 | | |
| 1·1 | 71 | 68 | 64 | 68 | | 71 | 71 | | |
| 0·5 | 71 | 69 | 65 | 73 | | 71 | 69 | | |
| Control | 71 | 71·5 | 69 | 74 | | 71 | 72 | | |
| | Solubility at 50° C. (Hannah) | | | | | | | | |
| 20·8 | 85 | 80 | 79 | 82 | 74 | 85 | 83 | 86 | 86 |
| 2·8 | 85 | 83 | 78 | 83 | 77 | 85 | 82 | 87 | 87 |
| 1·1 | 85 | 82 | 78 | 84 | 78 | 85 | 82 | 91 | 92 |
| 0·5 | 85 | 87 | 83 | 85 | 80 | 85 | 85 | 91 | 91 |
| Control | 85 | 85 | 85 | 90 | 90 | 85 | 85 | 91 | 92 |

*Roller full-cream no. 2, 2·4 % moisture*

| Initial concentration of oxygen % | Days at 47° C. | | | | | Days at 37° C. | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 23 | 47 | 95 | 199 | 0 | 58 | 128 |
| | Solubility at 20° C. (Hannah) | | | | | | | |
| 20·8 | 79 | 78 | 77 | 76 | 70 | 79 | 81 | 81 |
| 5·6 | 79 | 76 | 77 | 75 | 71 | 79 | 79 | 78 |
| 1·6 | 79 | 79 | 81 | 80 | 71 | 79 | 80 | 84 |
| 0·4 | 79 | 81 | 79 | 77 | 72 | 79 | 80 | 81 |
| Control | 79 | 78 | 81 | 79 | — | 79 | 79 | 82 |
| | Solubility at 20° C. (Lyons) | | | | | | | |
| 20·8 | 77 | 82 | 79 | | | | | |
| 5·6 | 77 | 80 | 78 | | | | | |
| 1·6 | 77 | 87 | 83 | | | | | |
| 0·4 | 77 | 78 | 83 | | | | | |
| Control | 77 | 81 | 86·5 | | | | | |
| | Solubility at 50° C. (Hannah) | | | | | | | |
| 20·8 | 91 | 89 | 90 | 83 | 74 | 91 | 93 | 91 |
| 5·6 | 91 | 93 | 92 | 87 | 80 | 91 | 96 | 90 |
| 1·6 | 91 | 92 | 92 | 87 | 83 | 91 | 95 | 95 |
| 0·4 | 91 | 92 | 95 | 86 | 81 | 91 | 96 | 94 |
| Control | 91 | 90 | 94 | 91 | — | 91 | 96 | 94 |

*Roller full-cream no. 3, 3·7 % moisture*

| Initial concentration of oxygen % | Days at 47° C. | | Days at 37° C. | | |
|---|---|---|---|---|---|
| | 0 | 61 | 0 | 61 | 119 |
| | Solubility at 20° C. (Hannah) | | | | |
| 20·8 | 71 | 58 | 71 | 69 | 68 |
| 0·05 | 71 | 68 | 71 | 73 | 66 |
| | Solubility at 50° C. (Hannah) | | | | |
| 20·8 | 86 | 58 | 86 | 79 | 80 |
| 0·05 | 86 | 70 | 86 | 86 | 84 |

Table 8 (*continued*)

*Roller separated, 4·0 % moisture*

| Initial concentration of oxygen % | Days at 47° C. | | | | Days at 37° C. | |
|---|---|---|---|---|---|---|
| | 0 | 29 | 62 | 131 | 0 | 63 |
| | Solubility at 20° C. (Hannah) | | | | | |
| 20·8 | 74 | 71 | 71 | 70 | 74 | 71 |
| 6·0 | 74 | 71 | 71 | 71 | 74 | 71 |
| 1·4 | 74 | 72 | 73 | 70 | 74 | 73 |
| 0·34 | 74 | 71 | 72 | 71 | 74 | 74 |
| 0·17 | 74 | 71 | 72 | 70 | 74 | 72 |
| Control | 74 | 72 | 75 | 74 | 74 | 74 |
| | Solubility at 20° C. (Lyons) | | | | | |
| 20·8 | 72 | 71 | 69 | | 72 | 71 |
| 6·0 | 72 | 71 | 70 | | 72 | 71 |
| 1·4 | 72 | 72 | 71 | | 72 | 72 |
| 0·34 | 72 | 72 | 67 | | 72 | 73 |
| 0·17 | 72 | 71 | 70 | | 72 | 72 |
| Control | 72 | 73 | 73·5 | | 72 | 73 |
| | Solubility at 50° C. (Hannah) | | | | | |
| 20·8 | 90 | 89 | 83 | 78 | 90 | 91 |
| 6·0 | 90 | 91 | 86 | 83 | 90 | 91 |
| 1·4 | 90 | 89 | 89 | 85 | 90 | 92 |
| 0·34 | 90 | 87 | 88 | 85 | 90 | 92 |
| 0·17 | 90 | 89 | 87 | 86 | 90 | 93 |
| Control | 90 | 90 | 91 | 93 | 90 | 93 |

*Spray full-cream no. 1, 1·3 % moisture*

| Initial concentration of oxygen % | Days at 47° C. | | | | | | Days at 37° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 35 | 59 | 128 | 169 | 196 | 0 | 59 | 140 |
| | Solubility at 20° C. (Hannah) | | | | | | | | |
| 20·8 | 98 | 100 | 99 | 97 | 97 | 96 | 98 | 100 | 99 |
| 6·4 | 98 | 98 | 100 | 97 | 96 | 97 | 98 | 99 | 99 |
| 3·3 | 98 | 100 | 99 | 97 | 98 | 97 | 98 | 98 | 100 |
| 1·9 | 98 | 97 | 99 | 98 | 98 | 95 | 98 | 99 | 97 |
| Control | 98 | 100 | 100 | 98 | 98 | 96 | 98 | 99 | 99 |
| | Solubility at 20° C. (Lyons) | | | | | | | | |
| 20·8 | 99 | 99 | 97 | | | | 99 | 99 | |
| 6·4 | 99 | 99 | 98 | | | | 99 | 99 | |
| 3·3 | 99 | 99 | 98 | | | | 99 | 99 | |
| 1·9 | 99 | 99 | 97 | | | | 99 | 99 | |
| Control | 99 | 99 | 99 | | | | 99 | 98·5 | |
| | Solubility at 50° C. (Hannah) | | | | | | | | |
| 20·8 | 100 | 99 | 99 | 99 | 98 | 99 | 100 | 99 | 100 |
| 6·4 | 100 | 99 | 100 | 99 | 99 | 99 | 100 | 100 | 99 |
| 3·3 | 100 | 98 | 99 | 99 | 99 | 99 | 100 | 99 | 100 |
| 1·9 | 100 | 100 | 100 | 98 | 100 | 99 | 100 | 99 | 99 |
| Control | 100 | 98 | 100 | 99 | 99 | 100 | 100 | 99 | 100 |

A different method was adopted for the independent determinations of the solubility at 20° C. carried out on some of the powders at the laboratories of Messrs J. Lyons and Co., Ltd.

## Discussion

A temperature coefficient of 4 for a rise of 10° C. implies an acceleration of the production of insolubility by approximately 21 and 84 times respectively for storage at 37 and 47° C., as compared with a 'normal' atmospheric temperature of 15° C. On this basis there

would be no significant loss in solubility, whether determined at 20 or at 50° C., in the spray powders during storage at 15° C. for 45 years. Even if the temperature coefficient were to be so low as 2·5, which is very unlikely, the powder could still be stored for 10 years at 15° C. without appreciable loss in solubility.

In the case of the roller powders, which contained rather higher proportions of moisture, appreciable losses in solubility could be detected above the random spread in the figures due to experimental error in the determination. Two main points emerge from consideration of these data. First, the loss in solubility tends to be significantly less in the gas-packed than in the air-packed samples, indicating that oxygen, or perhaps changes produced in the milk by the action of oxygen (e.g. oxidation of the fat with production of peroxides, acids, etc.) favours the production of insolubility of the casein. The effect is most clearly brought out with roller full-cream powder no. 3 which, owing presumably to its high moisture content (3·7 %), suffered a relatively rapid reduction in solubility.

The second point meriting consideration is the consistently greater loss in solubility recorded when the determination was made at 50° C. than when it was carried out at 20° C., the difference being frequently of the order of 100 %. On very prolonged storage the excess of solubility at 50 over that at 20° C. therefore tended to be reduced, and in one case (roller full-cream no. 3) actually disappeared.

From the table it can be seen that the average loss in solubility in gas-packed roller full-cream powders containing less than 3 % of moisture (or separated powders containing less than 4 %) during storage for 1·5 months at 47° C. or 6 months at 37° C. is very small indeed, probably only of the order of 3–4 %. Assuming a temperature coefficient of 4 for 10° C., both of these times are equivalent to storage for 10 years at 15° C. Even after 6–7 months at 47° C. the loss in solubility of the gas-packed powders was only of the order of 10 %.

SUMMARY OF PART III

Solubility tests have been made on whole and separated dried milk samples which have been packed in air and in different concentrations of oxygen and stored at various temperatures. It has been concluded that changes in solubility due to storage at ordinary temperatures for a number of years will be exceedingly slight with gas-packed powders of reasonably low moisture content.

## PART IV. THE TECHNIQUE OF INERT GAS PACKING

C. H. LEA AND T. MORAN

The replacement of the air inside a gas-tight container by an inert gas, e.g. nitrogen, is, in theory, a relatively simple matter, the most convenient method being to remove as large a proportion of the air as practicable by means of a pump, and then to admit the inert gas until the container is once more at atmospheric pressure. Assuming, for example, that the pump employed reduces the pressure to 2 in. of mercury, then one, two or three of these cycles should reduce the oxygen content from 20·8 % (air) to 1·4, 0·09, or 0·006 % respectively. The use of still lower pressures greatly increases the efficiency of the process, and one or two cycles at 2 mm. should reduce the concentration of oxygen in the free space inside the container to 0·06 % or $1{\cdot}4 \times 10^{-4}$ % respectively.

In practice slight leakages of air into the apparatus and the presence of appreciable amounts of oxygen in the 'inert' gas prevent such low figures from being attained. Further,

when cans containing food material are treated in considerable numbers at a time, as must usually be the case, other difficulties arise.

The present paper deals with the experimental gas packing of milk powder with the object of achieving concentrations of oxygen sufficiently low to retard or inhibit tallowiness (cf. Part I).

### Design of a gas-packing unit

As already pointed out, exhausting to 2 in. pressure (a 28 in. vacuum) and refilling to atmospheric pressure with pure nitrogen should reduce the concentration of oxygen in a can originally full of air from 20·8 to approximately 1·4 %, and does so, provided that the volume of the connecting tubing used is negligible. If, however, the can, pierced with a small brogue hole, is placed inside a very much larger vessel, and the latter evacuated and refilled with nitrogen, it can be calculated (assuming rapid mixing of the incoming gas) that the concentration of oxygen obtained inside the can will be as high as 5·1 %, a figure

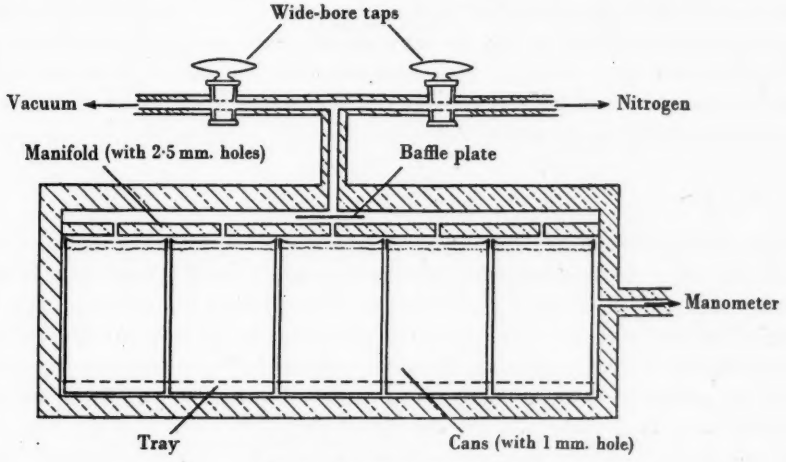


Fig. 7. Cabinet for gas-packing.

which was confirmed experimentally when a small can was filled inside a very large desiccator. This high figure is due to the fact that the composition of the gas in the larger vessel, i.e. the composition of the gas entering the can, changes only gradually from 20·8 to 1·4 % oxygen as the nitrogen is admitted.

It follows, therefore, that in a cabinet containing a number of cans and a considerable amount of free space, individual cans may tend to receive greater or smaller proportions of the residual oxygen from the free space according to their position in the cabinet relative to the point at which the nitrogen enters, and a poor degree of uniformity within the batch may result. Obviously, for efficient and economical packing the volume of the free space in a gas-packing cabinet should be reduced to an absolute minimum and, if practicable, the inlet for the gas should be so arranged that the advancing wave-front of gas covers the brogue holes in the cans as quickly and as uniformly as possible.

In Fig. 7 is shown a rough diagrammatic representation of a small experimental cabinet for twenty-five cans, designed according to these principles. The cabinet is fitted with a vacuum-pressure gauge, as well as a small mercury-in-glass manometer. The cans,

filled with milk powder to within 1 cm. of the top and pierced with a brogue hole in the centre of the lid, are introduced into the cabinet on a square tray. The front of the cabinet is then closed, the seal being made by means of a rubber gasket, and the cabinet exhausted. Nitrogen is introduced via a manifold pierced with twenty-five holes, one over each can, until the pressure slightly exceeds atmospheric. The front of the cabinet is then removed, the cans withdrawn and the brogue holes soldered, this operation for the whole twenty-five being easily completed in 1 min. Under these conditions no detectable leakage of air into the cans occurs. If, however, a larger number of cans must be treated in one batch, involving a more serious delay in soldering, it might prove advisable to apply a positive pressure to the powder for a little while before opening the cabinet in order that the cans might be soldered while still 'breathing'. As a means of still further reducing the free space within the cabinet, lengths of rod of square cross-section may be inserted between the cans.

The importance of even distribution of the incoming inert gas in a gas-packing unit is greatly reduced when the amount of free space in the cabinet is relatively very small, and the vacuum employed high, so that the amount of residual oxygen which could enter the cans from the free space is small. Such conditions would obtain in a cabinet designed for gassing one or a small number of large rectangular-sided cans which fit the cabinet, leaving only a negligible amount of free space.

### Gas-tightness of the container

The type of container used for storing milk powder in these experiments was the ordinary sanitary can made from (unlacquered) tin plate, in which the side seam is soldered and the ends rolled on. Such a can has proved very satisfactory commercially for a great variety of packing purposes, but it was recognized that in the case of milk powder the requirements are unusually exacting, in that the period of storage desired is long, and the standard of gas-tightness high. Further, in a can filled with a dry powder no self-sealing of small leaks by fluid contents of the can is possible.

The end seams of all cans used in the experiments were therefore protected by a continuous film of Wood's metal, which was applied by rotating the rim of the can in the metal melted under a layer of lactic acid (as flux), followed by washing with water. This procedure, which could be carried out very rapidly, produced, in effect, an all-metal seal, which has proved completely gas-tight. Precautions of this nature were considered necessary to ensure that no oxygen other than that originally present in the can could possibly gain access to the powder, otherwise erroneous conclusions might have been drawn.

A few cans sealed under vacuum but not treated with Wood's metal have shown no appreciable leakage during a period of 6–12 months. On the other hand, evidence from other sources indicates that under such circumstances leakage occasionally does occur. No dogmatic statement can therefore be made, but it seems very probable that with careful adjustment of the seaming machine, and particularly if use is made of one of the special vacuum sealing compounds now available, a can sufficiently gas-tight for commercial use should be obtained. Alternatively, of course, an all-soldered can may be used.

ANALYSIS OF THE HEAD-SPACE GAS IN CANS OF MILK POWDER

The apparatus (Fig. 8) employed for sampling the gas present in cans of milk powder consisted essentially of a perforated cone of silver steel carried on a wooden handle, the point being protected by a rubber stopper which, when well greased, made a gas-tight connexion to the end of the can. In use the rubber was held firmly against the top of the tin by means of the wooden handle, and the tube connecting the point to the gas burette exhausted by means of an oil pump. The tap to the pump was then closed, the point forced into the can, the tap of the burette opened, the mercury adjusted to the mark *A* near the top of the burette, and the pressure in the system read off. The volume of the connecting tube (*ca.* 5 ml.) being known, the pressure in the can could be calculated. Prior to examination the cans were immersed in a water-bath to attain a standard temperature.

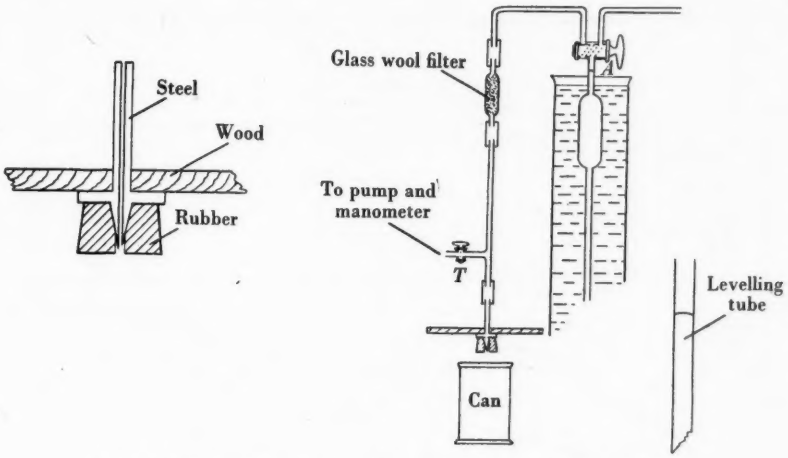


Fig. 8. Apparatus for measuring the pressure in cans and for sampling the head-space gas for analysis.

The Haldane apparatus employed for analysis of the gas was of the usual type, the burette being of 21 ml. gross capacity with 15 ml. in the bulb. Errors due to fluctuation of temperature were minimized by using a sample approximating very closely to the size of the compensating tube (20 ml.) and by bubbling air through the water in the jacket in order to maintain compensator and burette at exactly the same temperature.

*Types of milk powder*

Commercially prepared milk powders fall into two classes according to whether they have been prepared by drying in a thin film on heated drums (roller process), or by spraying into a current of hot air (spray process). Certain differences between these powders have already been discussed, namely, in solubility (Part III), and in facility of extraction of the fat by solvents (Part I). It has now been shown that during gas-packing the powders behave in a characteristically different manner.

In the drying of milk by the roller process the solid is removed from the surface of the drum in the form of a thin shred-like sheet, which is then ground and sifted to the desired

degree of fineness. The product consists of small flakes containing very few bubbles of entrapped gas. Spray-dried powder, on the other hand, whether produced by the pressure-spray or centrifugal-spray process, consists of small particles of a more spherical shape which under the microscope can be seen to enclose numerous bubbles of gas. This gas cannot be displaced by a fluid—for example, to obtain a true density for the solid material of a spray powder it is necessary first to take up in water and redry—and it was not known how much resistance they would oppose to removal under vacuum.

### Packing of roller-dried powders

The gas-packing of roller-process powders, whether full-cream or separated, presented no particular difficulty. In the small experimental cabinet charged with twenty-five cans of powder the pressure could be rapidly reduced to 3 mm. by the pump, but could only with

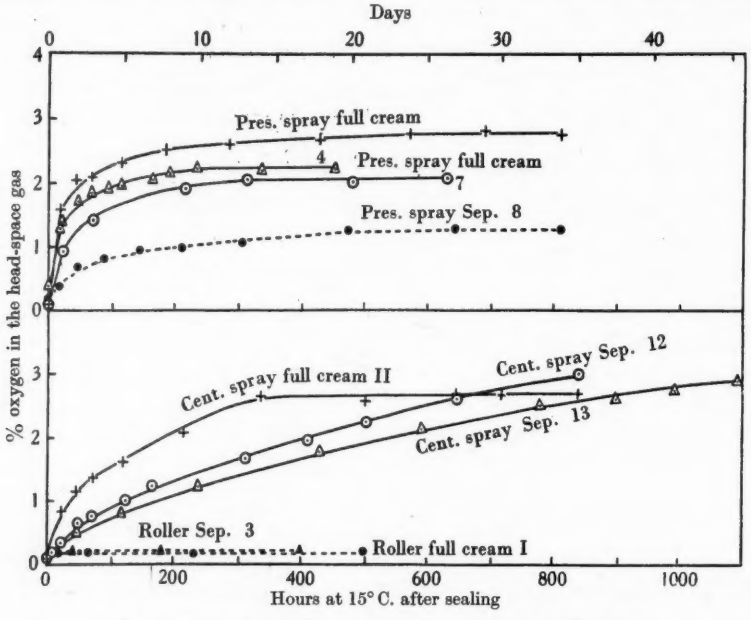


Fig. 9. 'Desorption' of oxygen from various types of milk powder.

difficulty be induced to fall beyond this point. A considerable part of the residual pressure was no doubt due to the vapour pressure of the moisture which was usually present to the extent of about 3 % in the powder. Under such conditions and employing 'oxygen-free' nitrogen containing less than 0·01 % of oxygen, one cycle reduced the oxygen content of the head-space gas (determined immediately after sealing) to about 0·1 %. After standing for 24 hr. this value was invariably found to have increased slightly, but thereafter remained practically constant for several weeks (Fig. 9).

The small 'desorption' of oxygen from roller powders during the first 24 hr. after packing (Table 9) must be due to the escape of entrapped or dissolved air, but it is obvious that such air must be very much smaller in amount and much less firmly held than in the case of spray powders. Regassing a sample of gas-packed roller full-cream powder 22 hr. after the first sealing gave a product in which no free oxygen could be detected (Table 11).

Table 9. *The gas-packing of milk powders*

| Powder | Type | Moisture content % | Wt. in 315 ml. can g. | Total* gas space in can ml./g. of powder | Time of exhausting min. | Lowest pressure reached mm. | % oxygen in head-space gas | | Gas within the particles ml./g. | Oxygen within the particles ml./g. at N.T.P. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Initial | Final | | |
| 1 | Roller full-cream | 2·6 | 110 | 2·087 | 2 | 3 | 0·09 | 0·20 | 0·0111 | 0·0021 |
| 2 | Roller full-cream | 3·7 | 101 | 2·344 | 3 | 3 | 0·08 | 0·23 | 0·0170 | 0·0033 |
| 3 | Roller separated | 2·5 | 130 | 1·729 | 3 | 3 | 0·10 | 0·25 | 0·0125 | 0·0024 |
| 4 | Pressure-spray full-cream | 1·3 | 156 | 1·244 | 3 | 3 | 0·20 | 2·25 | 0·1239 | 0·0240 |
| 5 | Pressure-spray full-cream | 1·4 | 175 | 1·024 | 5 | 2 | 0·12 | 1·63 | 0·0748 | 0·0145 |
| 6 | Pressure-spray full-cream | 1·7 | 175 | 1·024 | 3 | 3 | 0·20 | 2·80 | 0·1294 | 0·0251 |
| 7 | Pressure-spray full-cream | 1·8 | 175 | 1·024 | 2 | 2 | 0·10 | 2·08 | 0·0980 | 0·0190 |
| 8 | Pressure-spray separated | 2·0 | 183 | 1·027 | 3 | 3 | 0·18 | 1·28 | 0·0548 | 0·0106 |
| 9 | Centrifugal-spray full-cream | 1·9 | 135 | 1·560 | 2 | 2 | 0·11 | 5·40 | 0·3992 | 0·0774 |
| 10 | Centrifugal-spray full-cream | 2·3 | 175 | 1·024 | 5 | 2 | 0·10 | 3·30 | 0·1586 | 0·0307 |
| 11 | Centrifugal-spray full-cream | 2·6 | 175 | 1·024 | 3 | 2 | 0·13 | 2·70 | 0·1275 | 0·0247 |
| 12 | Centrifugal-spray separated | 3·3 | 150 | 1·406 | 3 | 2 | 0·10 | 3·10† | 0·2038† | 0·0395† |
| 13 | Centrifugal-spray separated | 3·9 | 150 | 1·406 | 3 | 3 | 0·15 | 3·20† | 0·2078† | 0·0403† |

* Head-space and space between and within the particles.

† Desorption still not quite complete (Fig. 9) and values therefore slightly low.

Table 10. *Effect of duration of exhaust and age of powder on the amount of oxygen remaining within the particles*

| Powder | Type | Age days | Moisture content % | Wt. in 315 ml. can g. | Total gas space in can ml./g. of powder | Time of exhausting min. | Lowest pressure reached mm. | % oxygen in head-space gas | | Oxygen within the particles ml./g. at N.T.P. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Initial | Final | |
| 1 | Roller full-cream | 1 | 2·6 | 110 | 2·087 | 2 | 3 | 0·09 | 0·20 | 0·0021 |
| | | | | | | 5 | 3 | 0·05 | 0·15 | 0·0019 |
| | | | | | | 15 | 2 | 0·00 | 0·09 | 0·0017 |
| | | | | | | 60 | 2 | 0·00 | 0·06 | 0·0012 |
| 7 | Pressure-spray full-cream | 2 | 1·8 | 175 | 1·024 | 2 | 2 | 0·10 | 2·08 | 0·0190 |
| | | | | | | 5 | 2 | 0·13 | 2·06 | 0·0187 |
| | | | | | | 10 | 2 | 0·05 | 2·00 | 0·0187 |
| | | | | | | 15 | 2 | 0·05 | 1·94 | 0·0181 |
| | | | | | | 60 | 2 | 0·10 | 1·94 | 0·0176 |
| 9 | Centrifugal-spray full-cream | 1 | 1·9 | 135 | 1·560 | 2 | 2 | 0·11 | 5·40 | 0·0774 |
| | | | | | | 5 | 2 | 0·10 | 5·40 | 0·0773 |
| | | | | | | 15 | 1·5 | 0·06 | 5·30 | 0·0764 |
| | | | | | | 60 | 1·5 | 0·03 | 5·20 | 0·0752 |
| 5 | Pressure-spray full-cream | 1 | 1·4 | 175 | 1·024 | 5 | 2 | 0·12 | 1·63 | 0·0145 |
| | | 3 | | | | 5 | 1·5 | 0·09 | 1·77 | 0·0161 |
| | | 7 | | | | 5 | 1·5 | 0·07 | 1·80 | 0·0166 |
| 10 | Centrifugal-spray full-cream | 1 | 2·3 | 175 | 1·024 | 5 | 2 | 0·10 | 3·30 | 0·0307 |
| | | 7 | | | | 5 | 2 | 0·10 | 3·60 | 0·0345 |

### Packing of spray-dried powders

Milk powders produced by two modifications of the spray-drying process have been examined. The 'Pressure-spray' powder was dried by forcing milk, precondensed in vacuum pans, through a small jet into a blast of hot air. The 'Centrifugal' product was produced by feeding the milk, precondensed in a climbing film evaporator, on to a concave disk revolving at a high speed in the top of the drying chamber. Powders of the two types were very similar in general properties, but differed appreciably in their behaviour on gas-packing.

Packing was carried out in the manner already described for roller powders. The cabinet charged with twenty-five cans of spray-dried powder could be evacuated to 2 mm. without difficulty, and 'initial' gas analyses carried out as soon as possible after sealing the cans gave values of from 0·1 to 0·2 % of oxygen. On standing, however, the oxygen content of the head-space gas increased, rapidly at first and then more and more slowly to a maximum value which varied in the different cases from 1·3 to 5·4 % (Table 9), after which it began very slowly to decrease as a result of the normal absorption of oxygen by the powder. This latter process is very slow at 15° C. under a low pressure of oxygen, and the samples were not usually retained sufficiently long for any appreciable fall in oxygen concentration to be observed. The powders listed in Table 9 were packed on the first or, in one or two cases, on the second day after manufacture.

Determination of the density of the solid matter of several samples of milk powders gave an average figure of 1·29 for full-cream powders and 1·44 g./c.c. for separated powders, roller- and spray-process powders giving the same result when the latter had been taken up in water and redried to the original moisture content in order to eliminate entrapped gas. By use of these values the total free space in the can could be calculated and, in conjunction with the initial and final (equilibrium) oxygen concentrations, an estimate of the amount of oxygen actually entrapped *within* the particles could be made (Tables 9–11).

*Effect of time of exhausting.* Since the solid material of a spray-dried power is not completely impermeable to gas, as shown by the fact that on storage equilibrium is ultimately established between the gas inside and outside the particles, it was thought that it might be possible to withdraw part or all of the entrapped gas during packing if the vacuum were applied for a time considerably in excess of the 2 min. required to remove the gas from between the particles.

Cans of several samples of powder were therefore exhausted for periods ranging up to 1 hr., filled with nitrogen, and the amount of oxygen still remaining within the particles determined in the usual way by plotting the 'desorption' curve. A series of curves of almost identical shape was obtained, the maximum values reached showing that, in fact, very little of the entrapped oxygen can be drawn off from a spray-dried powder by submission to a high vacuum (2 mm. pressure) even for as long as 1 hr. (Table 10).

*Effect of age of the powder.* It was further considered possible that the permeability of the substance of the particles of milk powder to oxygen might change rapidly with increasing age of the powder, as a result, for example, of the crystallization of films of vitreous lactose. Desorption curves were therefore determined on several powders at intervals of from 1 to 7 days after manufacture, the powder being stored at 15° C. in sealed tins to prevent absorption of moisture. Each portion of the powder was gas-packed

in precisely the same way, i.e. exhausting for 5 min. to a pressure of 2 mm. and refilling with pure nitrogen.

The curves obtained (Figs. 10, 11) showed the rather unexpected result that the amount of oxygen remaining in the particles after exhausting for 5 min. was actually greater in the stale than in the fresh powder (Table 10). There is therefore no evidence of any increase with age in the permeability of the particles to gas, so long as absorption of moisture is prevented. The effect of moisture has not been investigated, since milk powder prior to packing should not be stored under conditions which permit the moisture content to increase.

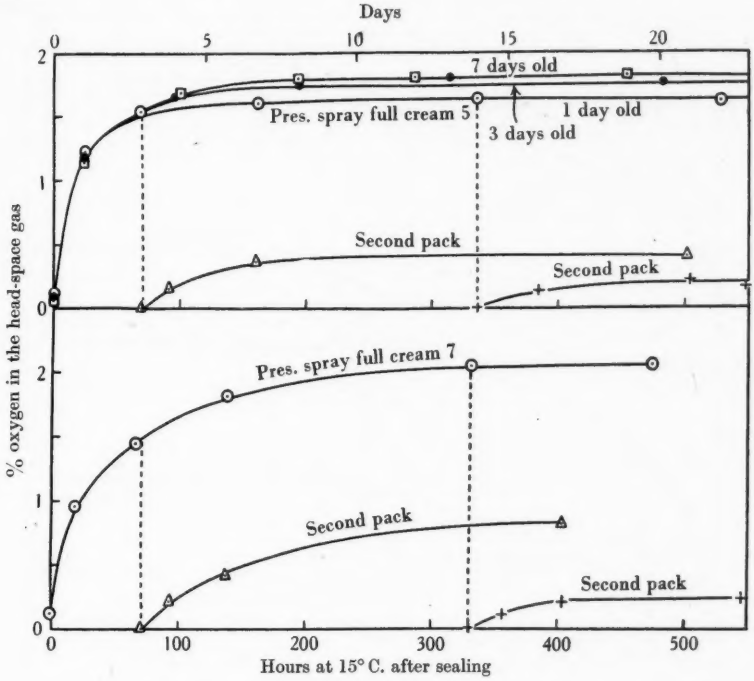


Fig. 10. Double packing of pressure-spray powders.

In explanation of the increased desorption of oxygen from the older powder it may be suggested that the air entrapped within the particles during drying is either somewhat depleted in oxygen owing to combination with the powder, or (perhaps more probably) is simply at a lower pressure due to cooling. The oxygen content of the 'pockets' in the powder may therefore still be increasing several days after manufacture of the powder, as the pockets approach equilibrium with the air.

It has not been practicable to compare powder immediately after manufacture with powder 1 day old, and it is possible that a very appreciable change might occur within the first 24 hr. This is a point in favour of packing in inert gas at the earliest possible moment after manufacture.

*Multiple-stage packing.* It is obvious from the results already quoted that even when a gas-tight cabinet, a good vacuum and pure nitrogen are used, resulting in an 'initial'

concentration of oxygen in the free-space gas of less than 0·1 %, the particles of a spray-dried power will still retain sufficient oxygen to raise the oxygen content of all the gas in the can to 2–3 % or even in some, perhaps exceptional cases, to 5 % or over.

The amount of entrapped oxygen in the powder therefore sets a limit beyond which single-stage packing cannot go, and if still lower concentrations are required it will be necessary to allow 'desorption' to proceed, either in part or to equilibrium, and then to puncture the can, draw off the gas and refill with nitrogen. An initial oxygen concentration of practically zero is usually obtained in this second treatment (Table 11, Figs. 10, 11),

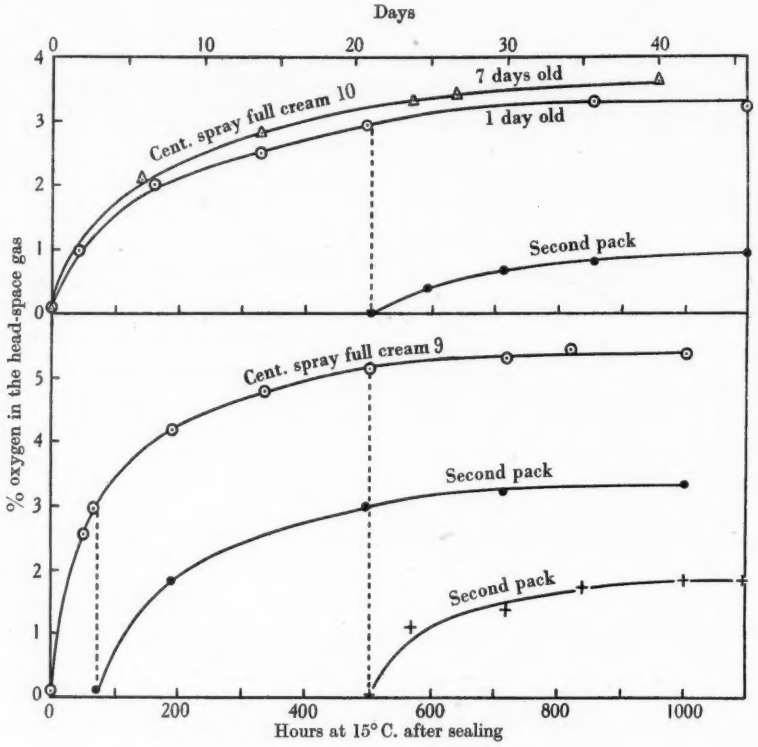


Fig. 11. Double packing of centrifugal spray powders.

increasing of course as a second but smaller phase of desorption sets in. A third treatment could be given if desired and the oxygen-content of the pack still further reduced, but in practice this should not be necessary.

### Effect of compression of milk powders

Milk powder contains a very large amount of air between the particles; in fact half to three-quarters of the space occupied by the powder is air, the precise proportion varying very considerably with the sample.

It appears therefore that compression of the powder might be used as a method of reducing the oxygen/powder ratio in a can, either as a supplement to or an alternative for

Table 11. *Effect of a second cycle in reducing the amount of oxygen retained within the particles*

| | | Oxygen in first pack | | | | Oxygen in second pack | | |
|---|---|---|---|---|---|---|---|---|
| Powder | Type | Initial % | Final % | ml./g. at N.T.P. within the particles | Re-gassed after hr. | Initial % | Final % | ml./g. at N.T.P. within the particles |
| 1 | Roller full-cream | 0·05 | 0·15 | 0·0019 | 22 | 0·00 | 0·00 | 0·0000 |
| 7 | Pressure-spray full-cream | 0·13 | 2·06 | 0·0187 | 72 | 0·00 | 0·80 | 0·0077 |
| | | | | | 331 | 0·00 | 0·24 | 0·0023 |
| 5 | Pressure-spray full-cream | 0·12 | 1·63 | 0·0145 | 70 | 0·00 | 0·40 | 0·0038 |
| | | | | | 336 | 0·00 | 0·20 | 0·0019 |
| 10 | Centrifugal-spray full-cream | 0·10 | 3·30 | 0·0307 | 505 | 0·00 | 0·90 | 0·0086 |
| 9 | Centrifugal-spray full-cream | 0·10 | 5·40 | 0·0773 | 72 | 0·12 | 3·30 | 0·0466 |
| | | | | | 504 | 0·05 | 1·80 | 0·0256 |

Table 12. *Effect of compression on milk powders*

| Roller full-cream | | | | Roller full-cream | | | | Spray full-cream | | | | Roller separated | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pressure lb./sq. in. | Gross volume ml./g. | Air content ml./g. | Oxygen content* ml./g. | Pressure lb./sq. in. | Gross volume ml./g. | Air content ml./g. | Oxygen content* ml./g. | Pressure lb./sq. in. | Gross volume ml./g. | Air content ml./g. | Oxygen content* ml./g. | Pressure lb./sq. in. | Gross volume ml./g. | Air content ml./g. | Oxygen content* ml./g. |
| — | — | — | — | 0† | 2·810 | 2·035 | 0·395 | 0† | 1·875 | 1·100 | 0·213 | — | — | — | — |
| 1 | 2·460 | 1·685 | 0·327 | 1 | 2·712 | 1·937 | 0·376 | 1 | 1·809 | 1·034 | 0·201 | 1 | 2·500 | 1·805 | 0·350 |
| — | — | — | — | 10 | 2·318 | 1·543 | 0·299 | 10 | 1·472 | 0·697 | 0·135 | — | — | — | — |
| 200 | 1·465 | 0·690 | 0·134 | 50 | 1·922 | 1·147 | 0·222 | 50 | 1·428 | 0·653 | 0·127 | 200 | 1·925 | 1·230 | 0·239 |
| 400 | 1·300 | 0·525 | 0·102 | 100 | 1·639 | 0·864 | 0·168 | 100 | 1·385 | 0·610 | 0·118 | 400 | 1·700 | 1·005 | 0·195 |
| 600 | 1·170 | 0·395 | 0·077 | 250 | 1·487 | 0·712 | 0·138 | 250 | 1·334 | 0·559 | 0·108 | 600 | 1·540 | 0·845 | 0·164 |
| 800 | 1·095 | 0·320 | 0·062 | 500 | 1·300 | 0·525 | 0·102 | 500 | 1·300 | 0·525 | 0·102 | 800 | 1·435 | 0·740 | 0·144 |
| 1000 | 1·045 | 0·270 | 0·052 | 750 | 1·221 | 0·446 | 0·086 | 750 | 1·260 | 0·485 | 0·094 | 1000 | 1·365 | 0·670 | 0·130 |
| 1200 | 1·010 | 0·235 | 0·046 | 1000 | 1·164 | 0·389 | 0·075 | 1000 | 1·244 | 0·469 | 0·091 | 1200 | 1·305 | 0·610 | 0·118 |
| 1400 | 0·983 | 0·208 | 0·040 | 1250 | 1·108 | 0·333 | 0·065 | 1250 | 1·235 | 0·460 | 0·089 | 1400 | 1·265 | 0·570 | 0·110 |
| 1600 | 0·960 | 0·185 | 0·036 | 1500 | 1·099 | 0·324 | 0·063 | 1500 | 1·218 | 0·443 | 0·086 | 1600 | 1·230 | 0·535 | 0·104 |
| 1800 | 0·943 | 0·168 | 0·033 | 1750 | 1·080 | 0·305 | 0·059 | 1750 | 1·213 | 0·438 | 0·085 | 1800 | 1·200 | 0·505 | 0·098 |
| 2000 | 0·930 | 0·155 | 0·030 | 2000 | 1·057 | 0·282 | 0·055 | 2000 | 1·192 | 0·417 | 0·081 | 2000 | 1·185 | 0·490 | 0·095 |

* Corrected from 20 to 0° C.

† Settled by lightly tapping the can, but not otherwise compressed.

gas-packing. The pressing of milk powder into blocks has already been proposed (20), but mainly as a means of reducing the rate of uptake of moisture from the atmosphere.

The data given in Table 12 show that the amount of air associated with 1 g. of milk powder can, in fact, be greatly reduced by compressing the powder, much of the improvement being achieved at relatively low pressures (Fig. 12). In the case of roller powder the improvement amounted to as much as 7–11 times—equivalent to reducing the oxygen content of the head-space gas from 20·8 to 2–3 %—but in spray powder only to 2½ times, the lower figure presumably being due to the initially closer packing of the uncompressed spray powder, and to the air cells in the interior of the particles.

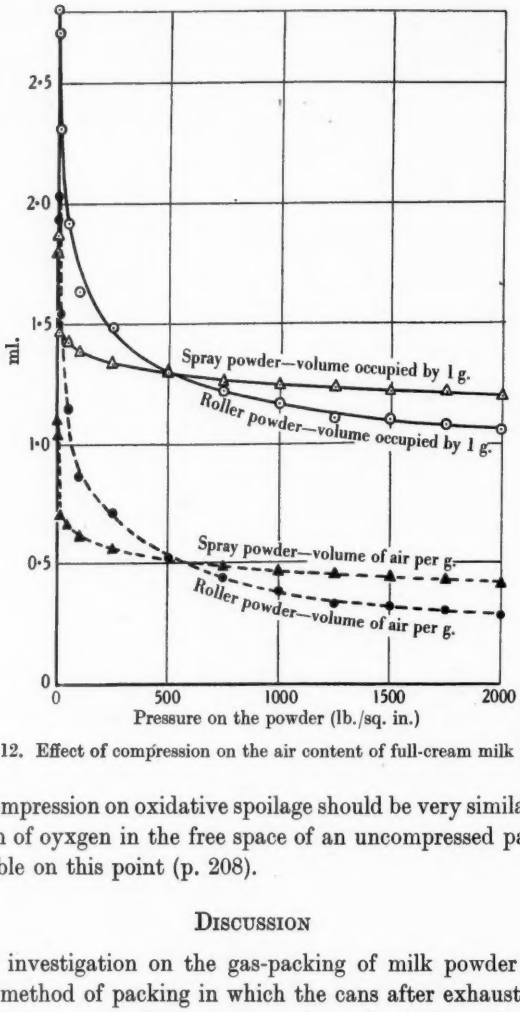


Fig. 12. Effect of compression on the air content of full-cream milk powders.

The effect of compression on oxidative spoilage should be very similar to that of reducing the concentration of oyxgen in the free space of an uncompressed pack. Some evidence is already available on this point (p. 208).

## Discussion

In the foregoing investigation on the gas-packing of milk powder attention has been confined to that method of packing in which the cans after exhausting are refilled with inert gas, and sealed at atmospheric pressure after removal from the cabinet.

A similar reduction of the oxygen/powder ratio in the can may, of course, be achieved without the use of inert gas by 'vacuum-packing', whereby the can is sealed inside the

cabinet while still under reduced pressure. Alternatively, the quantity of oxygen in the can might be still further reduced by exhausting, refilling with nitrogen, again exhausting and sealing under reduced pressure. Disadvantages attached to the use of these methods are the more complicated apparatus required with the accompanying difficulty of rendering it gas-tight, and the limitation imposed on the size of the can which must be able to withstand the pressure of the atmosphere.

Results obtained in Part II concerning flavour changes in powders packed in various concentrations of oxygen at atmospheric pressure can be extended to cover vacuum packing and the packing of compressed powders, provided that all data are converted to ml. of oxygen at N.T.P. per gram of powder before comparison is made. Even in comparing powders packed without compression under inert gas at atmospheric pressure the figures for percentage of oxygen in the head-space gas possess only a limited significance owing to the considerable variation in bulk density encountered even in milk powders of the same type.

Whichever method of packing is finally adopted, the use of a really high vacuum is of fundamental importance; it has already been pointed out (p. 184) that two cycles at 2 in. pressure are less effective than one at 2 mm., though requiring twice as much inert gas.

The milk powders examined have been found to vary very considerably both in the amount of oxygen entrapped within their particles and in the difficulty in removing it. Roller-dried powders could be packed in concentrations of oxygen nearly as low as those obtainable in 'empty' cans, and apart from a small increase of 0·1–0·2 % during the first 24 hr. after sealing, showed no further change.

Spray-dried powders were packed equally effectively, in that the air from between the particles was almost completely displaced, but the vacuum used failed to draw off a considerable quantity of gas from within the particles which slowly diffused into the head-space gas after the cans were sealed. The quantity of entrapped oxygen varied with the powder (Table 9), but was always greater than the 0·01 ml./g. of powder specified as the maximum quantity of oxygen permissible if all trace of talloviness is to be prevented (Part II). If, therefore, this very high standard is to be maintained, double packing must be resorted to in the case of spray-dried powders. For many purposes, however, in cases where the concentration obtained by single packing only exceeds the critical figure by two or three times, the additional protection afforded by double packing will not be considered to justify the extra cost.

In the series of powders investigated, those dried by the pressure-spray process tended on the whole to contain less entrapped oxygen and to release it more rapidly than the centrifugal-spray powders. This observation, however, by no means establishes the method of producing the spray as the critical factor, since some other difference in the procedure at the two factories concerned may equally well have been the cause. Thus batches of powder, even from the same factory, have been found to vary appreciably. In one extreme case, when the plant was started after a period of dislocation, a powder (no. 9) was obtained which was rather lighter and more voluminous than usual and contained two or three times the normal amount of entrapped oxygen. It is possible, therefore, that factors such as degree of precondensing as well as the actual production of the spray have a marked influence on the amount of air entrapped by the powder. Further investigation is necessary.

In the present paper multiple packing has been suggested as the simplest method of

removing entrapped oxygen. Since concluding the work our attention has been directed to a U.S. patent (21) in which a different principle is utilized. According to the procedure there described cans of spray-dried powder are exhausted to 1–2 mm. pressure in large cylindrical tanks, and allowed to stand under the vacuum for 20 hr., whereby the amount of oxygen retained by the powder is said to be considerably reduced and the keeping properties of the powder improved. Gas-tightness of the apparatus is ensured by the use, where necessary, of a solid paraffin-beeswax sealing compound. The lid, for example, is provided with a flange terminating in a pipe which dips beneath the surface of the wax carried in a channel at the top of the tank. Cold water or steam circulated through the pipe is used to make or break the seal.

### Summary of Part IV

Factors governing efficiency in gas-packing have been discussed, and a procedure described for reducing the oxygen concentration in cans of milk powder to any desired degree.

(*Pts. I–IV. MS. received for publication* 5 *October* 1939.)

## Part V. FURTHER OBSERVATIONS ON THE GAS-PACKING OF MILK POWDER, INCLUDING DATA ON STORAGE FOR PERIODS EXCEEDING THREE YEARS

C. H. LEA and J. A. B. SMITH

In the work described in the previous parts (Parts I–IV) it was attempted by means of accelerated tests extending over a few months at 47 and 37° C. to define the conditions necessary for the successful storage of full-cream milk powder for long periods at atmospheric temperature. The conclusions then reached have now been supplemented by observations on the behaviour of the same and other powders when stored in atmospheres containing various concentrations of oxygen for 3–4 years at 15° C. In general, the predictions made on the basis of the accelerated tests have been confirmed, and the conclusions extended.

### Changes in solubility

From the results of the accelerated tests described in Part III it was concluded that changes in the solubility of gas-packed milk powders of low moisture content due to storage under temperate conditions for a number of years should be very slight. Spray-dried milk powders containing less than 2 % of water should retain their solubility without appreciable loss for at least 10 years at 15° C., and probably for much longer, and any loss of solubility in roller-dried powder containing not more than 3 % of water (4 % for separated powder) should be very slight indeed, probably not more than 3–4 % in 10 years at 15° C.

The data now given in Table 13 show that up to 3 or 4 years these predictions are confirmed. The spray-dried powders, both full-cream and separated, show no loss of solubility, even when stored at 37° C. (98° F.).

Measurement of the solubility of roller-dried full-cream powders is difficult owing to the presence of fat together with a considerable proportion of insoluble material. Within the limit of the accuracy of the determination the results at 15° C. (59° F.) show no loss of solubility in any of the three roller full-cream powders, when gas-packed. Even no. 3, which contained 3·7 % of moisture, retained its solubility without loss for the full

3½ years of the experiment. In air there was no loss in roller full-cream powder no. 1, possibly a very slight loss in roller full-cream powder no. 3, and a small but definite loss in roller full-cream powder no. 2.

At 37° C. the gas-packed roller full-cream powders all showed some loss of solubility.

Table 13. *Changes in the solubility of milk powders during storage at* 15° C. (59° F.) *and at* 37° C. (98° F.)

| Powder | Moisture content % | Initial % oxygen in head-space gas* | Age at 15° C. months | Age at 37° C. months† | Solubility at 20° C. | Solubility at 50° C. |
|---|---|---|---|---|---|---|
| Spray full-cream no. 1 | 1·3 | 20·8 (air) | 46 | — | 98 | 99 |
| | | 6·4 | 46 | — | 99 | 99 |
| | | 3·3 | 46 | — | 98 | 99 |
| | | 1·9 | 46 | — | 98 | 99 |
| | | Control | — | — | 99 | 100 |
| | | Freshly made powder | | | 98 | 100 |
| | | 3·3 | — | 37 | 98 | 100 |
| | | 1·9 | — | 37 | 99 | 100 |
| Spray full-cream no. 2 | 1·3 | 20·8 (air) | 43 | — | 98 | 99 |
| | | 8·4 | 43 | — | 99 | 99 |
| | | 2·1 | 43 | — | 98 | 99 |
| | | 0·6 | 43 | — | 98 | 99 |
| | | 0·2 | 43 | — | 99 | 100 |
| | | Control | — | — | 99 | 99 |
| | | Freshly made powder | | | 98 | 99 |
| | | 2·1 | — | 36 | 98 | 100 |
| | | 0·6 | — | 36 | 100 | 100 |
| | | 0·2 | — | 36 | 99 | 99 |
| Spray separated | 1·4 | 20·8 (air) | 44 | — | 99 | 99 |
| | | 2·8 | 44 | — | 99 | 100 |
| | | 1·1 | 44 | — | 98 | 99 |
| | | 0·5 | 44 | — | 99 | 99 |
| | | Control | — | — | 99 | 100 |
| | | Freshly made powder | | | 99 | 99 |
| | | 1·1 | — | 46 | 100 | 100 |
| | | 0·5 | — | 46 | 100 | 100 |
| Roller full-cream no. 1 | 2·9 | 20·8 (air)* | 47 | — | 81 | 87 |
| | | 2·8 | 47 | — | 78 | 87 |
| | | 1·1 | 47 | — | 78 | 85 |
| | | 0·5 | 47 | — | 82 | 86 |
| | | Control | — | — | 78 | 88 |
| | | Freshly made powder | | | 75 | 85 |
| | | Control (average of 8 previous determinations) | | | 75 | 88 |
| | | 1·1 | — | 41 | — | 73 |
| | | 0·5 | — | 41 | — | 78 |
| | | Control for 37° samples | | | — | 86 |
| Roller full-cream no. 2 | 2·4 | 20·8 (air) | 45 | — | 69 | 85 |
| | | 5·6 | 45 | — | 76 | 87 |
| | | 1·6 | 45 | — | 84 | 91 |
| | | 0·4 | 45 | — | 82 | 91 |
| | | Control | — | — | 78 | 89 |
| | | Freshly made powder | | | 79 | 91 |
| | | Control (average of 6 previous determinations) | | | 80 | 92 |
| | | 1·6 | — | 39 | — | 79 |
| | | 0·4 | — | 39 | — | 81 |
| | | Control for 37° samples | | | — | 87 |
| Roller full-cream no. 3 | 3·7 | 20·8 (air) | 42 | — | 70 | 79 |
| | | 0·1 | 42 | — | 72 | 84 |
| | | Control | — | — | 71 | 81 |
| | | Freshly made powder | | | 71 | 86 |

* Density of packing of spray full-cream powders 0·50, of spray separated powder 0·58, and of roller full-cream powders 0·32 g. powder per ml. container volume.

† All the full-cream powders stored at 37° C. were held for a further 8–11 months at 0° C. before solubility was determined.

When examined after storage for 3 years at 37° C. the protein of roller powder no. 3 had obviously become completely insoluble, and the powder would not reconstitute to a milk-like fluid even in hot water. Gas-packed roller powders 1 and 2 had suffered a considerable* loss of solubility as shown by the figures determined at 50° C. The corresponding figures at 20° C. could not be obtained owing to some change in the powder which made reproducibility in the determination extremely unsatisfactory. The air-packed powders at 37° C. were not available for examination, having been discarded as inedible at a very early stage in the experiment.

### Changes in palatability

Milk powder from different factories, and frequently even from a particular factory at different times, varies appreciably in flavour. For purpose of the storage tests samples of each batch of powder stored were therefore gas-packed and held at −20° C. for use as 'controls' in the tasting tests, as a base-line from which deterioration could be measured. Where, as in the tests at 47 and 37° C., storage lasted only for a few weeks or months, this procedure was sound. When storage continued for a number of years the method became more open to criticism owing to the possibility of deterioration in the controls. Such deterioration, if it occurred at all, was, however, very slight, since on several occasions when a test was made the 'control' compared quite favourably with a fresh powder from the same factory. In any case spoilage might be expected to be more rapid at 15 than at −20° C., and absence of any significant difference between samples stored at the two temperatures should indicate absence of definite spoilage at either.†

The general method described in Part II has again been adhered to in the examination of the stored milks, except that the spray-dried powders were now usually reconstituted directly in water at 20° C. instead of in water at 60° C. followed by cooling to 20° C. The scale of marking was as described on p. 174. A hidden control was always included in the series, the sample considered best (not necessarily the control) being marked '0'. Milks averaging below 1 in general palatability were good, and for practical purposes can be considered as virtually unchanged. Samples averaging between 1 and 2 were showing slight deterioration, but were quite usable. Up to mark 2·5 the milk would probably be considered usable for cooking purposes, but above this point can be considered as definitely spoiled, samples averaging above 3 being unpleasant to taste.

The figures for general palatability and the averaged order of preference given in Table 14 confirm in general the findings of the accelerated tests. 'Off' flavour marks of between 1 and 2 occasionally recorded for the better gas-packed powders at 37° C. were awarded for 'heated' flavour, not for tallowiness. After storage for more than 3 years at 37° C. gas-packed roller powders 1 and 2 had acquired a definitely more 'heated' or condensed milk flavour than the controls, but were free from tallowiness or marked staleness and were still usable. The corresponding spray-dried powders, particularly nos. 2 and 3, were quite good.

The conclusions to be drawn from these and other data on palatability are considered later (p. 213).

* A solubility below about 80 % indicates complete insolubility of the casein, and below about 75 % complete insolubility of the protein.

† Statements in the literature to the effect that milk powders (not gas-packed) retain their flavour more satisfactorily at temperatures above 0° C. than below, may be due to storage in non-air-tight containers which permit absorption by the powder of the slight odour almost invariably associated with cold-storage rooms.

Table 14. *The effect of storage for long periods at 15 and 37° C. on the flavour of milk powders ('off' flavour mark, and averaged order of preference as recorded by the tasting panel)*

Spray full-cream no. 1, 1·3 % moisture

| Initial oxygen content % | Months storage at 15° C. 3 Mark | 5 Mark | 8 Mark | 10½ Mark | 10½ Order | 25½ Mark | 25½ Order | 43½ Mark | 43½ Order | Months at 37° C. 37 Mark | 37 Order |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20·8 (air) | 0·7 | 2·2 | 2·8 | 2·7 | 5·0 | 3·8 | 5·0 | 3·2 | 5·0 | 3·4 | 6·0 |
| 6·4 | — | — | — | 1·8 | 4·0 | 2·6 | 4·0 | 1·8 | 4·0 | 2·8 | 4·9 |
| 3·3 | — | — | — | 0·0 | 2·0 | 1·1 | 2·7 | 0·6 | 2·4 | 2·1 | 3·2 |
| 2·2 | — | — | — | — | — | — | — | — | — | 1·8 | 2·8 |
| 1·9 | — | — | — | 0·2 | 2·5 | 0·4 | 1·7 | 0·1 | 1·5 | 1·5 | 2·8 |
| Control | 0·0 | 0·0 | 0·0 | 0·0 | 1·5 | 0·3 | 1·6 | 0·4 | 2·1 | 0·6 | 1·3 |

Spray full-cream no. 2, 1·3 % moisture

| Initial oxygen content % | Months storage at 15° C. 2½ Mark | 4½ Mark | 7 Mark | 15 Mark | 15 Order | 21 Mark | 21 Order | 40½ Mark | 40½ Order | Months at 37° C. 37 Mark | 37 Order |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20·8 (air) | 0·8 | 2·0 | 2·2 | 2·5 | 3·0 | 3·7 | 6·0 | 3·8 | 6·0 | 3·9 | 7·0 |
| 8·4 | — | — | — | — | — | 2·4 | 5·0 | 2·4 | 5·0 | 2·6 | 6·0 |
| 2·1 | — | — | — | — | — | 1·1 | 2·9 | 0·3 | 1·9 | 1·3 | 4·9 |
| 0·6 | — | — | — | — | — | 1·1 | 2·9 | 0·8 | 3·2 | 0·1 | 2·2 |
| 0·5 | — | — | — | — | — | — | — | — | — | 0·1 | 2·5 |
| 0·2 | — | — | — | 0·0 | 1·5 | 0·8 | 2·5 | 0·6 | 2·7 | 0·2 | 2·6 |
| Control | 0·0 | 0·0 | 0·0 | 0·0 | 1·5 | 0·2 | 1·7 | 0·4 | 2·2 | 0·2 | 2·8 |

Spray separated no. 3, 1·4 % moisture

| Initial oxygen content % | Months storage at 15° C. 6 Mark | 6 Order | 23 Mark | 23 Order | 41½ Mark | 41½ Order | Months at 37° C. 41 Mark | 41 Order |
|---|---|---|---|---|---|---|---|---|
| 20·8 (air) | 0·2 | 1·6 | 3·3 | 5·0 | 2·9 | 5·0 | 3·3 | 6·0 |
| 6·2 | — | — | 1·7 | 3·9 | 1·5 | 4·0 | 1·9 | 5·0 |
| 1·1 | — | — | 0·7 | 2·8 | 0·5 | 2·5 | 0·8 | 3·0 |
| 0·6 | — | — | — | — | — | — | 0·4 | 2·2 |
| 0·5 | — | — | 0·1 | 1·8 | 0·0 | 1·5 | 0·9 | 3·3 |
| Control | 0·0 | 1·4 | 0·0 | 1·5 | 0·3 | 2·0 | 0·0 | 1·5 |

Roller full-cream no. 1, 2·9 % moisture

| Initial oxygen content % | Months storage at 15° C. 4 Mark | 6 Mark | 9 Mark | 12 Mark | 15 Mark | 22½ Mark | 22½ Order | 27 Mark | 27 Order | 44½ Mark | 44½ Order | Months at 37° C. 41 Mark | 41 Order |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20·8 (air) | 1·3 | 0·9 | 2·5 | 2·3 | 2·8 | 3·7 | 5·0 | 3·8 | 5·0 | 3·9 | 5·0 | 4·0 | 5·0 |
| 2·8 | — | — | — | — | — | 1·7 | 4·0 | 1·9 | 3·9 | 1·0 | 3·1 | 1·9 | 3·5 |
| 1·1 | — | — | — | — | — | 0·7 | 2·6 | 0·7 | 2·3 | 0·5 | 2·2 | 1·6 | 3·1 |
| 0·5 | — | — | — | — | — | 0·2 | 1·7 | 0·3 | 1·9 | 0·7 | 2·4 | 1·4 | 2·4 |
| Control | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 1·6 | 0·2 | 1·8 | 0·6 | 2·3 | 0·0 | 1·0 |

Roller full-cream no. 2, 2·4 % moisture

| Initial oxygen content % | Months storage at 15° C. 3 Mark | 4½ Mark | 7 Mark | 7 Order | 10 Mark | 24½ Mark | 24½ Order | 43 Mark | 43 Order | Months at 37° C. 39 Mark | 39 Order |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20·8 (air) | 0·9 | 1·2 | 2·5 | 3·0 | 2·3 | 3·7 | 5·0 | 3·8 | 5·0 | 3·8 | 5·8 |
| 5·6 | — | — | — | — | — | 2·1 | 4·0 | 2·0 | 4·0 | 3·2 | 5·2 |
| 1·6 | — | — | — | — | — | 1·2 | 2·9 | 0·9 | 2·8 | 1·5 | 3·9 |
| 0·6 | — | — | — | — | — | — | — | — | — | 0·9 | 2·5 |
| 0·4 | — | — | 0·0 | 1·5 | — | 0·3 | 1·6 | 0·0 | 1·4 | 0·9 | 2·4 |
| Control | 0·0 | 0·0 | 0·0 | 1·5 | 0·0 | 0·2 | 1·5 | 0·3 | 1·8 | 0·2 | 1·2 |

Roller full-cream no. 3, 3·7 % moisture

| Initial oxygen content % | Months storage at 15° C. 2 Mark | 2 Order | 4 Mark | 4 Order | 7 Mark | 10½ Mark | 10½ Order | 40 Mark | 40 Order | |
|---|---|---|---|---|---|---|---|---|---|---|
| 20·8 (air) | 0·2 | 1·7 | 0·4 | 1·8 | 2·2 | 2·1 | 3·0 | 3·9 | 3·0 | Powder caramelized badly when stored at 37° C. for long periods |
| 0·1 | — | — | — | — | — | 0·0 | 1·5 | 0·3 | 1·7 | |
| Control | 0·0 | 1·3 | 0·0 | 1·2 | 0·0 | 0·0 | 1·5 | 0·0 | 1·3 | |

### Change in colour

A complete record of the colour of the various samples of milk powder during the whole of the storage period was not kept, but the few observations which were made suggest that the chief factors responsible for discoloration of the powder are high moisture content and high storage temperature. These factors being constant, darkening was more pronounced in air-packed than in gas-packed samples, but even practically pure nitrogen was unable to prevent serious darkening if the moisture content and temperature of storage were high.

Roller-dried full-cream powder no 3, of 3·7 % moisture content, was definitely brown in colour after storage for 1 month at 47° C. or 6 months at 37° C., whether in air or in nitrogen containing only 0·03 % of oxygen. After 2 months at 47° C. the powder was deep brown in colour and the sample stored in air had an unpleasant 'charred' or 'caramelized' odour. The gas-packed samples were nearly as dark in colour and had a definitely 'fishy' odour. All were completely inedible. The powder was in a similar condition when finally examined after storage for 3 years at 37° C. On the other hand, the same powder was still quite normal in colour after storage for 40 months at 15° C.

Roller-dried separated powder of 4·0 % moisture content (equivalent to 3·0 % on the full-cream powder) was definitely brownish in colour after storage for 4–5 months at 47° C., whether packed in air or nitrogen.

Roller-dried full-cream powder no. 1, of 2·9 % moisture content, showed definite slight browning in 5 months at 47° C. When finally examined after 41 months at 37° C. all the samples were slightly brown, and milk reconstituted from the powder was noticeably discoloured. After 44½ months at 15° C. all the samples were normal in colour.

Gas-packed roller full-cream powder no. 2, containing 2·4 % of moisture, showed only a very slight deepening in colour during storage for 5 months at 47° C. or 39 months at 37° C. The powder had a slightly 'burnt' aroma, but the milk when reconstituted was only very slightly deeper in colour than the control sample and was quite palatable. After 43 months at 15° C. the milk was normal in every way.

All three gas-packed spray-dried powders (moisture content 1·3–1·4 %) showed a just perceptible but negligible deepening in colour after 5 months at 47° C. or 3–4 years at 37° C. when compared with a control, but no change at 15° C. A number of other spray-dried powders containing from 1·5 to 2·3 % of moisture have been stored at 47° C. for 3 months without discoloration.

Fresh roller-dried powders usually tend to be yellower than the corresponding spray-dried products, and the same difference is noticeable in the reconstituted milks.

### The density of milk powders

Two values of great importance in the gas or vacuum packing of milk powder, and also in blocking are (*a*) the true density of the air-free milk solids, and (*b*) the apparent or bulk density of the powder or block. These determine the amount of storage space and of packaging material required for a given weight of milk solids and, for an air-tight pack, the amount of oxygen available for spoilage.

*The density of milk solids.* Before any calculation of the amount of oxygen inside a sealed container or block of milk powder can be made, a knowledge of the density of the

air-free solid material is required. This value cannot be determined directly on spray-dried powders which must first be taken up in water and redried in order to remove entrapped air from *within* the particles. In determining the density of milk powder by the usual pycnometer method, there is also some danger that small bubbles of air might remain between the particles, or that one or other constituent of the powder might be partially dissolved by the liquid used. The densities of air-free separated and full-cream milk powders have therefore been redetermined by a method which seems more suitable than the pycnometer method for powdered or granular material such as dried foods, which are liable to contain constituents soluble in most solvents.

The apparatus used consists of a flask of about 50 ml. capacity with ground-in stopper connected by glass capillary tubing via a three-way tap to (*a*) a two-stage oil pump, and (*b*) a 50 ml. gas burette and levelling tube containing mercury. In operation, the flask is nearly filled with a weighed quantity of powder and exhausted by the pump, a known volume of inert gas, suitably hydrogen or nitrogen, being in the burette. The flask is then connected to the burette by turning the three-way tap, the system rapidly adjusted to atmospheric pressure by means of the levelling tube, and the volume of gas remaining in the burette read off. The operation must be carried out quickly, and the reading taken before any appreciable solution or adsorption of the gas has been able to occur.

Table 15. *The density of air-free milk solids (g./ml.)*

| Separated milk | | | Full-cream milk | | |
|---|---|---|---|---|---|
| % fat | % water | Density | % fat | % water | Density |
| 0 | 0 | 1·465 | 26 | 1·5 | 1·32 |
| 1 | 0 | 1·46* | 26 | 2·0 | 1·32 |
| 1 | 2 | 1·45 | 26 | 3·0 | 1·31 |
| 1 | 3 | 1·44 | 26 | 3·5 | 1·31 |
| 1 | 4 | 1·44* | 27 | 1·5 | 1·32* |
| 2 | 2 | 1·45 | 27 | 2·0 | 1·31 |
| 2 | 3 | 1·44 | 27 | 3·0 | 1·31* |
| 2 | 4 | 1·44 | 27 | 3·5 | 1·31 |
| 2·4 | 1·4 | 1·45 | 28 | 1·5 | 1·31 |

* Determined experimentally: other values by calculation, assuming the density of butter fat to be 0·94 g./ml.

Table 15 gives the air-free densities of a series of milk powders of different compositions obtained partly by direct determination, partly by calculation. The values for separated powder agree well with the figure of 1·44 previously found by the pycnometer method, but the new values for full-cream powder (1·31–1·32) are appreciably higher than the figure of 1·29 previously obtained.

*The 'bulk density' of full-cream milk powder and of milk-powder blocks.* Uncompressed milk powders vary greatly in the volume they occupy per unit weight. Spray-dried powders are usually denser than roller powders, because the small spheres of spray powder pack together with less inclusion of air than do the small irregular plates of roller powder. Uncompressed spray-dried full-cream powders vary appreciably in bulk density according to the type of plant and degree of precondensation of the milk, but the usual range is from about 0·5 to 0·6 g./ml.* The density of uncompressed roller-dried powder is generally lower than that of spray powder, and varies between about 0·3 and 0·5 g./ml. The bulk

* The somewhat lower densities given in Table 17 represent powders packed in small cans with a head-space allowance of the order of 7–10 % for convenience in gas-packing.

density of roller powder is considerably influenced by the degree of fineness of grinding and can be increased by milling.

Under a hydraulic pressure of several tons per square inch full-cream milk powders of both types can be compressed to densities ranging up to 1·1–1·2 g./ml. without appreciable loss of fat, the lighter blocks being easily crumbled in the hand, but the denser ones requiring grinding in a mill before reconstitution.

Table 16 and Fig. 13 give data for the oxygen content of full-cream powder of air-free density 1·31 and bulk density ranging from 0·3 to 1·3 when packed in air or in atmospheres of inert gas containing from 1 to 4 % of oxygen. These figures show, for example, that an average sample of uncompressed spray-dried full-cream powder of bulk density 0·55 g./ml. gas-packed in an atmosphere containing 1 % of oxygen contains 0·01 ml. (N.T.P.) of oxygen per gram of powder, and is equivalent in this respect to a block of powder compressed (in air) to a density of 1·22–1·23 g./ml. Similarly, packs containing 2, 3 and 4 % of oxygen contain 0·02, 0·03 and 0·04 ml. (N.T.P.) of oxygen per gram of powder, and are equivalent to blocks of density 1·16, 1·09 and 1·03 g./ml.

Table 16. *The oxygen content of uncompressed or compressed milk powder of bulk density 0·3–1·3 g./ml. Packed in air or in inert gas containing 1–4 % of oxygen*

| Oxygen content of head-space gas %* | ml. oxygen (N.T.P.) per g. of powder† at various densities of packing (g. powder per ml. container or block volume) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0·30 | 0·35 | 0·40 | 0·45 | 0·50 | 0·55 | 0·60 | 0·70 |
| 20·85 (air) | 0·506 | 0·412 | 0·342 | 0·288 | 0·244 | 0·208 | 0·178 | 0·131 |
| 4·0 | 0·097 | 0·079 | 0·066 | 0·055 | 0·047 | 0·040 | 0·034 | 0·025 |
| 3·0 | 0·073 | 0·059 | 0·049 | 0·041 | 0·035 | 0·030 | 0·026 | 0·019 |
| 2·0 | 0·049 | 0·039 | 0·033 | 0·028 | 0·023 | 0·020 | 0·017 | 0·013 |
| 1·0 | 0·024 | 0·020 | 0·016 | 0·014 | 0·012 | 0·010 | 0·008 | 0·006 |

| Oxygen content of head-space gas %* | ml. oxygen (N.T.P.) per g. of powder† at various densities of packing (g. powder per ml. container or block volume) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0·80 | 0·90 | 1·00 | 1·05 | 1·10 | 1·15 | 1·20 | 1·25 |
| 20·85 (air) | 0·096 | 0·069 | 0·047 | 0·037 | 0·029 | 0·021 | 0·014 | 0·007 |
| 4·0 | 0·018 | 0·013 | 0·009 | 0·007 | 0·005 | 0·004 | 0·003 | 0·001 |
| 3·0 | 0·014 | 0·010 | 0·007 | 0·005 | 0·004 | 0·003 | 0·002 | 0·001 |
| 2·0 | 0·009 | 0·007 | 0·005 | 0·004 | 0·003 | 0·002 | 0·001 | 0·001 |
| 1·0 | 0·005 | 0·003 | 0·002 | 0·002 | 0·001 | 0·001 | 0·001 | — |

* Assumed at 17° C. and 760 mm. † To convert to mg. oxygen per g. of powder multiply by 1·429.

## The absorption and desorption of oxygen by milk powder

### *Absorption*

Preliminary data on the absorption of oxygen by milk powder during storage in air at 47, 37 and 15° C. have already been given (Part I, Fig. 2), the results being expressed simply in terms of the falling concentration of oxygen in the head-space gas in an air-tight can. Though this method shows the main result fairly clearly, it does not take into account the considerable differences in bulk density between the various powders and the gradually falling pressure inside the can as the oxygen is absorbed and a smaller quantity of carbon dioxide produced. Completed data in absolute units for the same six powders are given in Fig. 14.

Several points of difference between the behaviour of separated and full-cream powders and between roller- and spray-dried powders can be seen from these curves. The separated powders absorb oxygen at a slow and uniform rate, the only departure from linear

absorption being a slight falling off with the roller powder at 47° C. as the amount of oxygen remaining in the can becomes seriously reduced.

With the full-cream powders the **S**-shaped curve of fat oxidation is superimposed on the slower and regular absorption by the non-fatty solids. Once the rapid phase of fat oxidation has been reached, there is but little slackening in rate, even when absorption approaches the limit set by the quantity of oxygen available in the sealed container. At

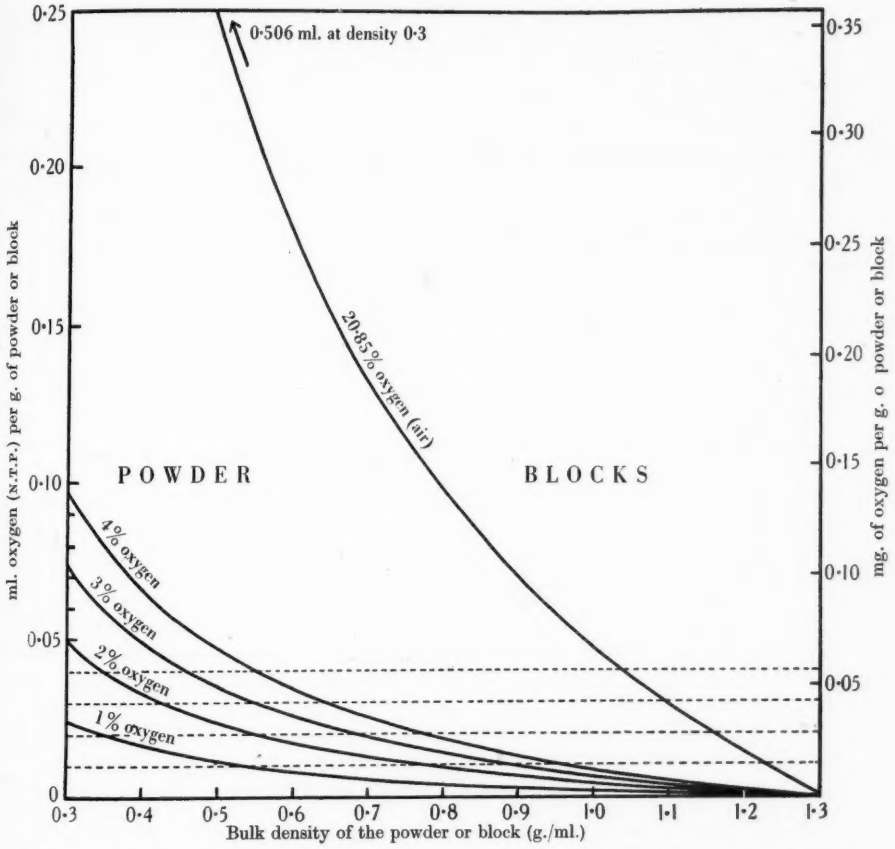


Fig. 13. The oxygen content of uncompressed or compressed milk powder of bulk density 0·3–1·3 packed in air or in atmospheres of inert gas containing 1–4 % of oxygen.

15° C. the roller-dried full-cream powders showed more clearly marked and longer induction periods than did the spray-dried products, an observation in agreement with the greater resistance to fat oxidation (cf. Part I, Fig. 5) and to the development of 'tallowy' flavours shown by this type of powder at normal atmospheric temperatures. At 37 and 47° C. the difference between the oxygen absorption curves of the roller- and spray-dried full-cream powders was less marked, in agreement with the observation that 'tallowy' flavour and chemical symptoms of fat oxidation (cf. Part I, Fig. 4) did, in fact, develop quite readily in roller-dried powders stored in air at these high temperatures.

The roller-dried separated powder (moisture 4·0 %, fat 1·0 %) provided the best example of oxidation of the non-fatty milk solids with minimal interference by fat, and it is of interest that in this case the temperature coefficient* of oxygen absorption was as high as 3·2 between 15 and 37° C., and 4·3 between 37 and 47° C. In the spray-dried powder (moisture 1·4 %, fat 2·4 %), which showed a temperature coefficient of 2·2–2·3

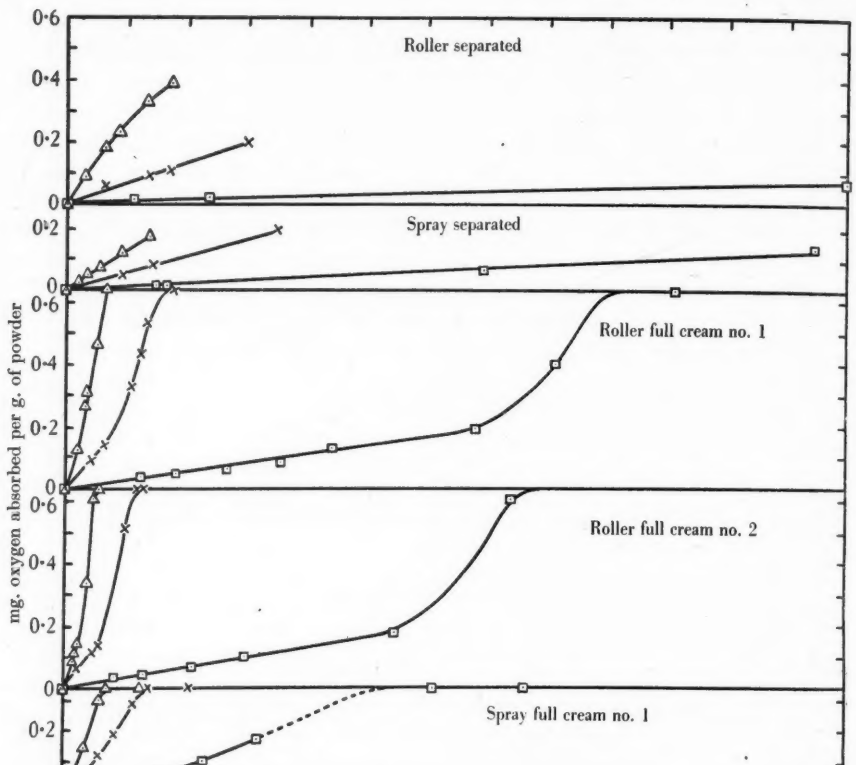


Fig. 14. The absorption of oxygen by various types of milk powder during storage at 47° (△), 37° (×) and 15° C. (⊡).

for both temperature ranges, fat was probably being oxidized, and this powder did in fact develop a distinctly 'tallowy' flavour after prolonged storage in air. The roller-dried full-cream powders showed temperature coefficients averaging about 2·5, and the spray full-cream powders about 2·0. Recent observations(22) on the development of peroxide in

* Defined as the ratio of the times necessary to produce a given degree of change at two temperatures 10° C. apart.

fats aerated at 97·7 and 110° C. yield a temperature coefficient of 2·1 for 10° C., a figure which agrees well with the present findings for spray-dried milk powder and with the approximate values already deduced in Part I for the development of 'tallowy' flavour in milk powder (p. 167).

*Desorption*

It has already been shown in Part IV that, after gas-packing to an initial oxygen content of 0·0–0·2 %, the percentage of oxygen in the free-space gas in a can of spray-dried milk powder gradually increases to a figure which may range from about 1 to over 5 %, according to the type and sample of powder. Some powders were found to desorb comparatively rapidly, the greater part of their oxygen being released within the first 3 days, and they soon approached equilibrium, usually at a figure of about 2 %. On the other hand, other powders evolved their oxygen more slowly and required a longer time to reach equilibrium, which was usually attained at a concentration of the order of 3 %. Powders dried by a 'pressure' or 'jet' spray process were found usually to belong to the first type; 'centrifugal' spray powders usually conformed to the second. The dividing line, however, was far from sharp, and much overlapping occurred, powders obtained at different times from the same factory varying very considerably in the amount of entrapped oxygen they contained.

Some further observations on desorption have now been made and these results, together with the earlier data, are summarized in Table 17. The formula derived to calculate the volume $V$ of the air space within the particles, as ml./g. of powder, was as follows:

$$V=\frac{(D-d)(C-c)}{Dd(20{\cdot}85-c)},$$

where $D$ = the density of the air-free powder (1·31 for full-cream, 1·44–1·45 for separated powder); $d$ = the density of packing (g. powder per ml. container volume); $c$ = the percentage of oxygen immediately after gas-packing; and $C$ = the percentage of oxygen after completion of desorption. In deriving this formula it was assumed that the cavities within the particles contain air (20·85 % oxygen) at atmospheric pressure. In freshly prepared powder either or both of these assumptions may be untrue, but the error is largely cancelled if the result is expressed as ml. *oxygen* per g. of powder. This oxygen value (corrected from 17 to 0° C.) is given in Table 17, and is equal to $0{\cdot}1944V$.*

From the table it can be seen that the two varieties of pressure spray powders examined (one of these from three different factories) tended to behave similarly, though the range of values observed was considerable. Centrifugal spray powders of types A and B also behaved fairly similarly.

Centrifugal A powder no. 9, which gave the highest desorption yet recorded, was also the lightest and most voluminous spray powder yet encountered, and had been produced under somewhat abnormal conditions, probably from precondensed milk of lower solids content than usual.

Appreciable day to day variations apparently sometimes exist in powder from the same plant. Thus powders 22, 23 and 24 were produced on consecutive days without any intentional modification of the process, but gave distinctly different desorption curves,

* A measure of $V$ could be obtained more directly by determining the apparent density of the powder $\theta$, by the method already described (p. 201). The volume of the air pockets per gram of powder would then be given by $V=1/\theta-1/D$.

Table 17. *The desorption of oxygen from spray-dried milk powders at* 15° C.

| Sample no.* | Process | Moisture content % | Days old when packed | Density of packing g./ml. container volume | Oxygen content of head-space gas after days | | | | | | Entrapped oxygen ml./g. N.T.P. powder‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0† | 3 | 7 | 14 | 28 | 42 or 56 | |
| F4 | Pressure A | 1·3 | 1 | 0·495 | 0·20 | 1·90 | 2·10 | 2·25 | 2·25 | — | 0·0242 |
| F6 | Pressure A | 1·7 | 1 | 0·555 | 0·20 | 2·15 | 2·45 | 2·65 | 2·80 | — | 0·0254 |
| F7 | Pressure A | 1·8 | 2 | 0·555 | 0·13 | 1·45 | 1·80 | 2·05 | 2·06 | — | 0·0188 |
| F5 | Pressure A | 1·4 | 1 | 0·555 | 0·12 | — | — | — | 1·63 | — | 0·0147 |
| F5 | Pressure A | 1·4 | 3 | 0·555 | 0·09 | — | — | — | 1·77 | — | 0·0163 |
| F5 | Pressure A | 1·4 | 7 | 0·555 | 0·07 | — | — | — | 1·80 | — | 0·0168 |
| S8 | Pressure A | 2·0 | 2 | 0·581 | 0·18 | 0·80 | 0·95 | 1·12 | 1·28 | — | 0·0106 |
| F | Pressure B (*a*) | 1·7 | 30 | 0·492 | 0·50 | — | — | 3·01 | 2·98 | — | 0·0304 |
| S | Pressure B (*b*) | 4·0 | 2 | 0·500 | 0·16 | 0·85 | 0·98 | 1·09 | 1·13 | — | 0·0119 |
| F9 | Centrifugal A | 1·9 | 1 | 0·429 | 0·10 | 3·00 | 4·04 | 4·80 | 5·32 | 5·40 | 0·0779 |
| F10 | Centrifugal A | 2·3 | 1 | 0·555 | 0·10 | 1·36 | 2·04 | 2·58 | 3·18 | 3·30 | 0·0312 |
| F10 | Centrifugal A | 2·3 | 7 | 0·555 | 0·10 | 1·56 | 2·20 | 2·83 | 3·43 | 3·60 | 0·0341 |
| F11 | Centrifugal A | 2·6 | 1 | 0·555 | 0·13 | 1·36 | 1·90 | 2·60 | 2·70 | — | 0·0250 |
| F14 | Centrifugal A | 1·6 | 0 | 0·555 | 0·15 | 1·25 | 2·00 | 2·65 | 3·35 | 3·65 | 0·0341 |
| F14 | Centrifugal A | 1·6 | 1 | 0·555 | 0·15 | — | — | — | — | 3·95 | 0·0371 |
| F14 | Centrifugal A | 1·6 | 3 | 0·555 | 0·12 | 2·10 | 2·90 | 3·35 | 3·85 | 4·17 | 0·0394 |
| F14 | Centrifugal A | 1·6 | 7 | 0·555 | 0·15 | — | — | — | — | 4·30 | 0·0405 |
| F22 | Centrifugal A | 2·1 | 4 | 0·483 | 0·34 | 2·60 | 2·85 | 2·95 | 3·10 | 3·00 | 0·0342 |
| F23 | Centrifugal A | — | 3 | 0·483 | 0·34 | 1·95 | 2·07 | 2·12 | 2·20 | 2·20 | 0·0231 |
| F24 | Centrifugal A | 2·1 | 2 | 0·483 | 0·34 | 1·90 | 2·40 | 2·80 | 3·10 | 3·20 | 0·0355 |
| F26 | Centrifugal A | 1·8 | 63 | 0·460 | 0·40 | — | — | 2·55 | 2·88 | 2·97 | 0·0345 |
| F27 | Centrifugal A | 1·9 | 64 | 0·460 | 0·40 | — | — | 2·91 | 3·30 | 3·20 | 0·0376 |
| S12 | Centrifugal A | 3·3 | 2 | 0·476 | 0·10 | 0·76 | 1·20 | 1·76 | 2·70 | 3·60§ | 0·0461 |
| S13 | Centrifugal A | 3·9 | 2 | 0·476 | 0·15 | 0·60 | 1·00 | 1·56 | 2·28 | 3·20§ | 0·0403 |
| F | Centrifugal B | 1·4 | 2 | 0·500 | 0·06 | 2·02 | 2·63 | 2·80 | 2·85 | — | 0·0323 |
| S | Centrifugal B | 2·1 | 2 | 0·500 | 0·04 | 0·98 | 1·52 | 2·10 | 2·68 | 3·30 | 0·0399 |

* F = full cream, S = separated powder.

† Initial oxygen concentrations above 0·2 % indicate that commercial nitrogen containing up to 0·3 % of oxygen was used for packing.

‡ Absorption of oxygen by the powder, usually at the rate of about 0·1 % per month, proceeds during as well as after desorption. This effect, which has not been corrected for in the table would increase the entrapped oxygen figures by about 0·001 when calculated after 1 month and by about 0·002 when calculated after 2 months.

§ Desorption still not quite complete.

nos. 22 and 23 conforming in type to the rapidly desorbing pressure spray kind of powder rather than to the more usual centrifugal type represented by no. 24.

Gane (private communication), examining a series of Centrifugal A powders prepared from precondensed milk of solids content varying from 32·5 to about 50 %, has recently found a marked and fairly progressive decrease in the amount of oxygen desorbed as the solids content of the milk dried increased. Degree of precondensation therefore plays an important part in determining desorption when the method of spraying is fixed. The particular process used, however, also influences the result, as shown by the fact that the degrees of precondensation used in preparation of the powders listed in Table 17 were as follows: Pressure A, 40–42 % milk solids; Pressure B, *ca.* 30 % in one plant and 30–35 % in two others; Centrifugal B, 45–50 %; and Centrifugal A, *ca.* 42 % (40–45 %). Here the effect of differences in the method of spraying (particle size or rate of evaporation?) completely obscured any relationship between degree of precondensation and desorption, the powders dried from precondensed milks of the highest solids content tending in fact to show the greatest desorption.

Comparison of the data for separated and full-cream powders given in Table 17 show that in some cases at least desorption is slower from separated than from the corresponding full-cream powder. The inference is that a constituent of both types of powder, probably the lactose, is responsible for the retention of air in relatively impermeable envelopes, which are made more rather than less permeable by the presence of fat. This property, however, is not possessed by sucrose: Pressure B sweetened spray-dried full-cream milk powder containing 40 % of sucrose has been found to desorb only about half as much oxygen as the corresponding unsweetened full-cream powder.

The results quoted in Table 17 were obtained from small (A $2\frac{1}{2}$ = 860 ml., E 1 = 405 ml., or A 1 = 315 ml.) cans packed in the laboratory. Large cans packed under commercial conditions behave similarly. Thus, in a factory experiment on the gas-packing of spray full-cream powder Pressure B at a bulk density of about 0·52 g./ml. in 21 lb. cans 1–4 days after manufacture, an average oxygen content of 2·8 % was obtained in the head-space gas when the cans were examined 2–3 months after packing to an initial oxygen content of about 0·6 %. These values correspond to the original presence of about 0·025 ml. of entrapped oxygen per gram in the powder.

Further work is required on the mechanism of the retention of oxygen by spray-dried powders and on possible means of reducing or preventing it. One or two preliminary experiments have suggested that packing the powder while still warm from the plant reduces the amount of entrapped oxygen retained by the powder to about half its 'cold' value, but exhausting the cabinet seems to take considerably longer when these very fresh powders are used, and for this reason the method may not prove practicable.

### THE KEEPING PROPERTIES OF GAS-PACKED POWDER ON SUBSEQUENT EXPOSURE TO AIR

On theoretical grounds milk powder, after long storage in the very restricted supply of oxygen present in a gas or vacuum pack or in a compressed block, might be expected to develop tallowiness more rapidly on subsequent exposure to the air than would the original fresh powder. This conclusion is suggested by the nature of the autoxidation of butter fat, whereby a relatively long period during which only very small quantities of oxygen

are absorbed and very slight changes in flavour occur leads up to a period of rapid absorption of oxygen and marked deterioration in flavour. Whilst the amount of oxygen present in a restricted oxygen pack should never be sufficient to allow the second phase of deterioration to develop, an appreciable proportion of the induction period might pass while the powder is in this state, with a consequent reduction of storage life on subsequent exposure to the air.

The evidence at present available suggests that any such reduction in stability is not great. Storage tests at the Hannah Dairy Research Institute have shown that a spray-dried full-cream milk powder stored in the form of 1 or 1¼ lb. blocks protected by moisture-resisting wrappers* remain in good condition for at least 12 months. The milk was also found to be quite good when stored in the block for 8 months, ground, and the resulting powder held in moisture-proof containers for several months longer.

Very few storage tests have been carried out on gas- or vacuum-packed powders after opening the tin, since there is no reason why such powder should be exposed to the air for any great length of time before use. In a recent test, spray-dried full-cream powder stored in atmospheres containing 1·0 and 4·0 % of oxygen for 3¼ years at 15° C. was found to be in unchanged condition 3 months after opening the tins, the 1·0 % sample being very good and the 4 % sample quite good, though slightly stale by comparison with the other. The tin should, of course, be of a type which permits reclosure to exclude moisture. The collar-band tin is probably the best for small packs and the lever lid with soldered tagger for large packs.

### *The gas-packing of old powder*

If the maximum benefit from gas-packing is to be obtained, it is obviously desirable that the powder used should be in the freshest possible condition, and evidence has already been produced that fresh powder retains less entrapped oxygen than powder several days old. A delay of 2 or 3 days between preparation and gas-packing would not, however, be expected to reduce appreciably the subsequent keeping properties at a particular oxygen concentration, and evidence has been obtained that it does not. Spray-dried full-cream powders have even been gas-packed in the laboratory 1 or 2 months after manufacture and found to keep quite well, but such a procedure is definitely not to be recommended.

## The technique of gas-packing

Gas-packing cabinets of the type described in Part IV have now been in use for several years for the packing of dried food products of various kinds for experimental purposes, and many thousands of cans ranging in size from 3 oz. to A2½ (860 ml.) have been gas-packed in them. While these simple cabinets have performed in a generally satisfactory manner, several minor refinements making for ease of operation or economy of inert gas might be incorporated in new pieces of apparatus of this type.

The volume of the gas-distributing system in the original cabinet is fairly small, but could be further reduced by replacing the manifold by three or more tubes each recessed into the top of the cabinet and pierced with three or more holes. A further small economy of gas would be effected by making the cabinet and sliding tray in the shape of a parallelogram with 60° and 120° angles instead of rectangular, thereby permitting closer packing of cylindrical cans. For ease and speed of operation the door of the cabinet, which carries

* Some of the blocks stored were prepared by Dr C. C. Thiel of the Dairy Section of the Council for Scientific and Industrial Research, Australia.

the rubber gasket, could be hinged at the top and counterpoised so that it will remain open. A further feature, which is useful when commencing the exhaust and when applying positive pressure before opening the cabinet, is a wedge type of door fastening which compresses the gasket without the aid of the vacuum. Several of these features are now available on commercial gas-packing units.

The use of a really good vacuum is of fundamental importance in gas-packing. A pump which reduces the pressure to 2 in. of mercury ('a 28 in. vacuum') should, with a perfect cabinet and pure nitrogen, reduce the oxygen content of the head-space gas from 20·8 to 1·4 %, whilst a pump which reduces the pressure to 2 mm. should, under similar conditions, reduce the oxygen content to 0·06 %. It is true that a second cycle with the less efficient pump can be given, but the consumption of time and gas is thereby doubled, and the efficiency of the second cycle will be very low unless a considerable time is allowed for the gas in the cans to mix after the first filling before the vacuum is again applied. Moreover, any slight leakage of air into the apparatus during exhausting would tend still further to reduce the efficiency of a second cycle. The vacuum should be applied relatively slowly, e.g. 10 in./min. for the first 2 min. in order to prevent bumping and possible choking

Table 18. *The effect of delay in soldering on the oxygen content of the gas in the can*

| Inert gas | Positive pressure treatment | Diameter of hole mm. | Increase in oxygen content (%) after | | |
|---|---|---|---|---|---|
| | | | 3 min. | 6 min. | 10 min. |
| Nitrogen | — | 1·2 | 0·15 | 0·27 | 0·46 |
| Nitrogen | — | 1·0 | 0·13 | 0·26 | 0·40 |
| Nitrogen | — | 0·6 | 0·10 | 0·20 | 0·36 |
| Nitrogen | — | 0·4 | 0·04 | 0·18 | 0·27 |
| 50 % nitrogen, 50 % carbon dioxide | — | 0·6 | — | 0·20 | 0·40 |
| 50 % nitrogen, 50 % carbon dioxide | 10 min. at 6 in. | 0·6 | 0·00 | 0·07 | 0·11 |
| 50 % nitrogen, 50 % carbon dioxide | 5 min. at 6 in. | 0·6 | 0·08 | 0·14 | 0·19 |

of the brogue hole with powder, which would also make soldering more difficult. With small cans no appreciable advantage is gained by maintaining the vacuum after the maximum attainable value has been reached, and exhausting should not require more than 5 min. in all. The nitrogen is then admitted fairly rapidly to slightly above atmospheric pressure, held there for ½–1 min., and the cabinet opened. A mercury manometer is much more suitable than the ordinary dial gauge for testing the performance of the pump and cabinet. The cabinet should not leak by more than the pressure of 1 mm. of mercury during the time required for admitting the nitrogen.

For soldering, the cans should be moved the least possible distance from the cabinet, the trays being slid in a horizontal plane, rather than lifted. Using an electric iron with a large bit or a gas or petrol iron one man can easily close 30–40 cans per minute.

Speed in soldering is essential because air is liable to enter the cans on standing (*a*) by diffusion and convection, or (*b*) through absorption by the powder of some of the gas in the can. Temperature effects, such as changes produced by the cooling of warm powder, are also liable to occur, but would probably be too slow to be of any practical importance.

The results given in Table 18 were obtained with A 2½ cans containing 1 lb. of spray-dried full-cream powder gas-packed several days after manufacture. As an alternative

method for automatic operation it might be possible to replace the brogue hole and solder method by application of a fraction only of the first process of seaming on the top, in order to hold the lid in position while exhausting and refilling with gas. The cans could then be fully closed by a machine after removal from the cabinet. In a trial in the laboratory using A $2\frac{1}{2}$ (860 ml.) cans full of roller-dried full-cream powder no difficulty was experienced in packing to the usual low initial content of oxygen, and leakage of oxygen into the cans during an interval of 10 min. before completion of the double seaming operation was only of the order of 0·4 %, i.e. approximately the same as through a 1 mm. brogue hole. This value was only slightly increased when the cans were caused to move slowly and smoothly during the 10 min. interval, as if on a belt. The method has not been further investigated.

### The use of carbon dioxide

In most of the storage experiments nitrogen has been used as the inert gas, either the 'oxygen-free' grade containing less than 0·01 % of oxygen or the cheaper product containing up to 0·3 % of oxygen. Mixtures of nitrogen and carbon dioxide may, however, be used, and have certain advantages for commercial operation. Carbon dioxide is absorbed more freely by full-cream powder than is nitrogen, probably because of the relatively high solubility of carbon dioxide in fat. By holding the powder in the cabinet under a pressure of carbon dioxide considerably greater than atmospheric for some time before opening to the air, a larger amount of the gas is absorbed by the powder than can be retained when the cans are withdrawn from the cabinet. The slow diffusion of this excess of carbon dioxide through the small brogue hole tends to prevent the diffusion of air back into the can during the interval between removal from the cabinet and soldering. This principle is utilized in an American patent (21) for gas-packing milk powder, in which the cans are held under carbon dioxide at a pressure of 10 lb./sq. in. (20 in. of mercury) above atmospheric for half an hour before opening to the air, whereby a 'safe' period of up to 30 min. is said to be obtained in which to solder the cans. The figures given in Table 18 show that the application of a positive pressure of as little as 3 lb./sq. in. (6 in. of mercury) for 10 min. to cans of milk powder packed under a mixture of equal volumes of nitrogen and carbon dioxide has some effect in slowing down leakage of oxygen into the cans, an effect which is not observed when nitrogen alone is used.

When gas containing a high proportion of carbon dioxide is used for packing full-cream powders, an appreciable diminution of pressure occurs after sealing, owing to partial absorption of this gas by the powder. The degree of concavity of the ends of cylindrical cans may then be used as a means of detecting and rejecting leaking cans. Milk powder packed in gas containing carbon dioxide has a slightly sharp taste when the tin is freshly opened, but this disappears very quickly on exposure to the air.

Experiments in progress show that a 50:50 mixture of nitrogen and carbon dioxide is just as effective as pure nitrogen in preventing the development of tallowiness in milk powder; reports in the literature to the effect that an atmosphere of carbon dioxide permits the development of tallowiness as rapidly as air could not be confirmed.

### Gas-packing in large containers

Gas-packing in large rectangular-sided containers is, in some respects, even simpler than in small cylindrical cans, in that waste space in the cabinet can be reduced to negligible proportions and no special care in distribution of the gas, is then necessary. Cabinets

made to take six '4 gallon' cans (*ca.* 21 lb. of spray-dried full-cream milk powder) have recently been employed successfully on a commercial scale for gas-packing dried milk). Care must be taken to avoid flexing the sides of these cans between opening the cabinet and soldering.

*An alternative method for gas- or vacuum-packing.* Gas-, or more particularly vacuum-packing in open-top cans, may also be carried out on an automatic line by bringing each can of powder in turn under a hood, applying a vacuum and seaming on the top while the can is still under the hood. Cans may be packed in this way at 40 or more per minute without being touched by hand. The degree of vacuum reached in the exhaust stage is usually considerably less good than that obtainable by the cabinet method, and with a light material such as milk powder there seems some danger of loss of powder by 'bumping' during the very rapid exhaust.

A modification of this process which is employed commercially for the gas packing of ground coffee(23) might, however, be suitable for milk powder. The machine used has twenty vacuum-pressure bells round a vertical axis, with a movable table under each. These tables raise and lower the can and lid into or out of the bell by cam action. The whole device rotates, discharging the sealed cans when complete. Filled cans with covers in place are fed on to the table and raised into the bells where the covers are lifted by magnets. High vacuum is applied for 8 sec. followed by 4 lb./sq. in. positive pressure of inert gas for 4 sec. A second application of vacuum lasts 11 sec. followed by a second application of positive pressure for 4 sec. The cans are sealed at a pressure only very slightly above atmospheric. Free oxygen is said to be almost completely removed from the cans by this method. No experience can be claimed with automatic gas-packing equipment of this kind.

*The gas-tightness of containers.* Little difficulty should be experienced in manufacturing and closing cylindrical open-top cans of up to 1 l. capacity so that the great majority should be gas-tight, and this is in fact established commercial practice, particularly in the U.S.A. However, an automatic testing device used in conjunction with inert gas containing carbon dioxide would be useful for rejecting the occasional leaky can. Alternatively, application of a suitable lacquer to the seams of the cans has been found an adequate substitute for the Wood's metal employed in the earlier experimental work for ensuring 100 % gas-tightness in open-top cans. An unsuitable lacquer may taint the contents of the can.

To obtain gas-tightness in large (21 lb.) tin-plate containers, together with sufficient mechanical strength to stand the hazards of transport, is a problem of greater difficulty, which, however, now seems to be practically solved.

## Discussion

*The quality of full-cream milk powder.* Fresh, spray-dried full-cream milk powder can be a very palatable product, and some commercial specimens reconstitute to a fluid which would pass even a critical taster as a 'rich' sample of fresh milk—an indication of what can be done with a good milk supply, an efficient plant and sufficient care in its operation. Usually, however, the product, though palatable, is fairly easily distinguished from fresh milk owing to its more 'creamy' or slightly 'heated' or 'condensed milk' taste. Occasionally it has a distinctive flavour suggestive of an early stage of tallowiness before this has

become at all unpleasant. Such incipient 'tallowy' flavours are possibly due in some, but probably not in all, cases to the use of copper equipment in the plant.

Usually, too, spray-dried full-cream milk reconstituted with *cold* water contains a small proportion of undissolved material which can be seen as particles in the film present on the walls of a glass vessel containing the milk, though the fluid itself should be quite 'smooth' on the palate. Only a poor sample tastes 'chalky'. This slight imperfection of solubility varies in degree with the particular drying process and operating temperatures used, and can be very slight. It is, in any case, eliminated almost completely by reconstitution with warm water. When used in tea or coffee a slight insoluble deposit sometimes remains at the bottom of the cup.

Notwithstanding these slight differences from fresh milk, spray-dried milk powder of good quality is an excellent substitute for fresh milk for general use.

Roller-dried full-cream powder, owing to its low solubility in cold water and much more 'heated' and frequently slightly 'stale' flavour, is less suitable than the spray product for use as liquid milk. It is quite suitable for many cooking purposes and is widely used for infant feeding.

*The optimal concentration of moisture for gas storage.* The moisture content of milk powder is mainly of importance in that a high moisture content favours loss of solubility, discoloration and the development of stale and fishy flavours during storage. Since the reactions producing insolubility and (probably) discoloration have high temperature coefficients, the maximum permissible moisture content will be lower when the powder is to be submitted to tropical temperatures than when storage is to be under temperate conditions only.

Gas-packed spray-dried full-cream powders containing 1·3, 1·6, 1·7, 1·8 and 1·9 % of moisture have been found to keep sufficiently well at 37° C., and for a few months even at 47° C., to suggest that such powders should withstand tropical temperatures* for considerable periods without serious deterioration. Where a moisture content below 2 % is obtainable without appreciable loss of solubility or increase in 'heated' taste, as is certainly the case with some commercial spray-drying plants, this would seem to be a desirable feature productive of optimal keeping properties under difficult conditions of storage. On the other hand, a moisture content of 2·5 % should be satisfactory for very long periods under temperate conditions, and 3 % is not too high for moderate temperatures and periods of storage.

The experimental data for roller-dried full-cream powders indicated satisfactory storage for 3½ years at a constant temperature of 15° C. (59° F.) when the moisture content of the powder was as high as 3·7 %. At 37° C., however, powders containing as little as 2·4 % of moisture suffered some loss of solubility when stored for long periods, powder containing 2·9 % of moisture showed definite discoloration as well as loss of solubility, and powder containing 3·7 % of moisture was very much worse in both respects and became quite unusable. Since 15° C. would be exceeded for an appreciable portion of the year, even in temperate climates, and since the harmful effect of exposure to temperatures above the mean would more than counterbalance the beneficial effect of periods below the mean, it would seem desirable for optimal keeping properties in gas packs to

* The mean annual temperature of Massawa on the Red Sea, one of the hottest places in the world, is about 30° C., the monthly mean ranging from 25·6° C. in January to 34·4° C. in July, and the mean daily maximum from 29·4 to 39·5° C.

maintain the moisture content of roller full-cream powder at 3 % (equivalent to 4 % on the separated powder) or below, and probably at a still lower figure if prolonged exposure to tropical conditions is likely.

*The effect of long storage in inert gas on palatability.* Although packing in inert gas was found in the accelerated tests at 47 and 37° C. to be effective in retarding or preventing the development of tallowiness in milk powder (Part II), an optimistic estimate of the probable storage life of the gas-packed powder at ordinary temperatures could not be given, because some doubt existed as to whether the accelerated tests which had been used successfully to predict the development of tallowiness could be applied also to the development of 'stale' flavours due (probably) to changes in the protein. Although such flavours were never definitely observed in spray full-cream powders of low moisture content that were not already 'tallowy', the tasting results showed apparent signs of their development in the roller powders after storage for a few months at all temperatures, including even −20° C. Subsequent observations have, however, discredited this finding, which was apparently due to an increasing criticism by the tasting panel of the slight 'stale' flavour already present in the fresh roller powder, a criticism which increased as the standard of the panel was raised by concentrating on the more palatable spray-dried powders. Gas-packed roller full-cream powders after storage for several years at 15° C. have, in fact, been found to be as palatable as fresh samples of powder obtained from the factory for comparison, and a similar observation has been made with several samples of spray full-cream and separated powders. While it cannot be claimed that gas packing, even under the best conditions, *completely* eliminates deterioration in the flavour of full-cream milk powder, it certainly reduces spoilage to negligible proportions over very long periods of storage.

A further point which might be mentioned in this connexion is the fact that freshly opened tins of gas-packed milk powder sometimes possess a slight odour which has been described as 'fruity'. This defect has been particularly observed in packs of initially low oxygen content which have been stored for very long periods until all the oxygen originally present in the can has been absorbed. The flavour of the reconstituted milk usually seems to be unaffected.

*The critical concentration of oxygen.* Reduction of the amount of oxygen in a can of gas- or vacuum-packed milk powder has two effects: (*a*) it reduces the *rate* of oxidation, thereby extending the time required for the powder to develop a given degree of 'off' flavour; and (*b*) it limits the degree of 'off' flavour that can ultimately be reached.

Factor (*a*) is mainly of importance during the first 12–18 months of storage. After a period ranging from 12 to about 36 months at 15° C. all the free oxygen in an air-tight container of powder, whether air- or gas-packed, has been used up. Deterioration in flavour then ceases, in fact when the quantity of oxygen originally present has been relatively small, e.g. a gas-pack containing about 4 % of oxygen, maximal deterioration in flavour appears to be reached before all the free oxygen has been absorbed.

On continued storage in the absence of free oxygen a tendency towards an improvement in flavour is sometimes observed, particularly in gas-packed powders which had undergone only very limited deterioration. Thus a powder packed in inert gas containing 3 or 4 % of oxygen may taste slightly better after 3 or 4 years' storage than it did when only 12 or 18 months old. Such changes are, however, quite small, and never suffice to make a badly deteriorated powder good again. Thus, in powder packed in an air-tight container

but not gas-packed the 'off' odour and flavour gradually changes in character, becoming less typically 'tallowy', but still remaining unpalatable. This change in flavour is presumably a result of the disappearance of reactive aldehydes and peroxides which, as shown by the fall in Kreis and peroxide values, begins to occur even before all the free oxygen has been consumed and continues more or less rapidly after its disappearance (Part I). Milk powders stored in non-air-tight containers, e.g. in lever-lid tins, are usually even more objectionable after very prolonged storage than powders which have been stored in air-tight tins, as was the case with the air packed powders in the present series of experiments.

## Summary

The following *summary of the effect of increasing quantities of oxygen in producing 'off' flavour in gas-packed full-cream milk powder during storage for long periods* has been based on observations made in the present series of experiments, supplemented to some extent by results from later work on the gas storage of spray-dried powder which is not here reported in detail.

(*a*) Full-cream powders stored in the presence of up to 0·01 ml. of oxygen per g. of powder kept very well at both normal and high temperatures. This figure corresponds to 1 % of oxygen (after completion of desorption) in the free-space gas of a can of spray-dried powder packed to a bulk density of 0·55 g./ml., or to 0·5 % of oxygen in a can of roller powder packed to a bulk density of 0·35 g./ml. Tallowiness was never definitely detected under such conditions, and there seems to be little or no advantage to be gained, at least so far as palatability is concerned, by improving on this figure. An atmosphere containing not more than 0·01 ml. of oxygen per g. of powder can therefore be considered an ideal pack for milk powder.

(*b*) Powders stored in the presence of 0·02 ml. of oxygen per g., i.e. approximately 2 % of oxygen in the free-space gas of a can of spray-dried full-cream powder, also kept well, in fact when storage was at 15° C. it was often impossible to distinguish between such powder and others stored in the presence of much smaller amounts of oxygen. At the high temperature of 37° C. the powder was usually but not invariably distinguishable from powders stored in lower concentrations of oxygen, but the difference was slight. Below 0·02 ml. of oxygen per g. of powder may therefore be considered a good commercial pack.

(*c*) Powder stored in the presence of 0·03 ml. of oxygen per g., i.e. approximately 3 % of oxygen in the free-space gas of a can of spray-dried full-cream powder, kept quite well. The tasting panel could usually distinguish between powder stored for long periods at 15° C. in this and in lower concentrations of oxygen, but the difference was still small and probably not important from a practical point of view. In view of the difficulty of gas-packing spray-dried powders in very low concentrations of oxygen, values up to 0·03 ml. of oxygen per g. of powder can be accepted as satisfactory for a commercial pack. It should be possible to store full-cream powder under these conditions for several years without serious deterioration. A rather lower concentration of oxygen than 0·03 ml./g. might, however, be desirable if storage is to include exposure to high atmospheric temperatures.

(*d*) Powder stored in the presence of 0·04 ml. of oxygen per g., i.e. approximately 4 % oxygen in the free-space gas of a can of spray-dried full-cream powder, developed a suspicion of 'off' flavour (mark 1·0) in about 7 months and a definite slight 'tallowy' odour and flavour ('off' flavour mark 1·5–2·0) in about 12 months at 15° C., but was little, if any,

worse after 2 or 3 years' storage. Though always considered usable and sometimes quite good, such powder was definitely inferior to samples stored in lower concentrations of oxygen, and so high a figure as 0·04 ml./g. should not be accepted as satisfactory. At 37° C. results were more unfavourable than at 15° C., and a sample of spray full-cream powder stored at this temperature was considered to have become unusable for part of the storage period, between 4 and 12 months, when 'off' flavour marks between 2·0 and 2·8 were recorded. The powder subsequently improved slightly to mark 2·0 at 12 and 16 months.

The authors wish to express their cordial appreciation of the assistance afforded to them by Mr F. D. Farrow and Mr C. J. Bushrod of the Metal Box Co. Research Department, who co-operated in packing the powders used for the storage experiments and throughout the work have given the benefit of their wide knowledge and experience in this field. Grateful thanks are also due to Dr L. H. Lampitt and the Research Staff of Messrs J. Lyons and Co., Ltd., for valuable assistance in the arduous task of assessing the flavour and solubility of the numerous samples of powder examined.

## REFERENCES

(1) HUNZIKER, O. F. (1935). *Condensed Milk and Milk Powder*, 5th edition, p. 555. Illinois, U.S.A.: published by the author.
(2) HUNZIKER, O. F. (1935). *Condensed Milk and Milk Powder*, 5th edition, p. 057. Illinois, U.S.A.: published by the author.
(3) SOMMER, H. H. (1923). *Proc. World's Dairy Congress, Lond.*, pp. 974, 981.
(4) DAVIES, W. L. & GILL, E. (1936). *J. Soc. chem. Ind. Lond.* **55**, 141T.
(5) HOLM, G. E. & GREENBANK, G. R. (1923). *Proc. World's Dairy Congress, Washington*, **2**, 1253.
(6) SUPPLEE, G. C. (1923). *Proc. World's Dairy Congress, Washington*, **2**, 1248.
(7) LEA, C. H. (1938). *Rancidity in Edible Fats* (Special Report No. 46 of the Food Investigation Board, H.M. Stationery Office), p. 138.
(8) LAMPITT, L. H. & BUSHILL, J. H. (1931). *J. Soc. chem. Ind. Lond.* **60**, 45T.
(9) STEBNITZ, V. C. & SOMMER, H. H. (1937). *Oil and Soap*, **24**, 228.
(10) LEA, C. H. (1929). *Rep. Food Invest. Bd Lond.* p. 30; (1931), *Proc. Roy. Soc.* **108**B, 175; (1938), *Rancidity in Edible Fats*, p. 107.
(11) WALTERS, W. P., MUERS, M. M. & ANDERSON, E. B. (1938). *J. Soc. chem. Ind. Lond.* **57**, 53.
(12) WECKEL, K. G. & JACKSON, H. C. (1936). *Food Res.* **1**, 419.
(13) MOHR, W. (1937). *Proc. 11th World's Dairy Congress, Berlin*, **3**, 348.
(14) WRIGHT, N. C. (1932). *J. Dairy Res.* **4**, 122.
(15) MOHR, W. & RITTERHOFF, K. (1937). *Proc. 11th World's Dairy Congress, Berlin*, **2**, 249.
(16) CLAUSEN, P. (1937). *Proc. 11th World's Dairy Congress, Berlin*, **2**, 233.
(17) SUPPLEE, G. C. & BELLIS, B. (1925). *J. Dairy Sci.* **8**, 39.
(18) Report of Committee on Remade Milk (1923), quoted by Allen, L. A. (1932). *Bull. Hannah Dairy Res. Inst.* (The Properties of Milk in Relation to Condensing and Drying). No. 3.
(19) HOWAT, G. R., SMITH, J. A. B., WAITE, R. & WRIGHT, N. C. (1939). *J. Dairy Res.* **10**, 498.
(20) KELLNER, E. (1937). *Proc. 11th World's Dairy Congress, Berlin* (quoted by Hunziker, O. F. (1937), *Dairy Industr.* **2**, 341).
(21) MERRELL, I. S. & SCHIBSTED, H. (1930). U.S. Pat. 1,786,858. 30. 12. 1930.
(22) MEHLENBACHER, V. C. (1942). *Oil and Soap*, **19**, 137.
(23) ANON. (1940), *Food Industr.* **12**, 39.

*Pt. V. MS received for publication, 30 December* 1942.

612.392.84

# REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

## SECTION D. THE NUTRITIONAL VALUE OF MILK AND MILK PRODUCTS

CONTENTS


I. Introduction

II. General

III. Nutritional value as estimated in laboratory and field experiments
- A. Raw milk (and butter in the cases of vitamins A and D)
  - (*a*) General
  - (*b*) Vitamins:
    - (i) General
    - (ii) Vitamin A and carotene
    - (iii) The vitamin B complex
      - Vitamin $B_1$
      - Riboflavin
      - Nicotinic acid
      - Pantothenic acid
      - Vitamin $B_6$
      - Biotin
      - Inositol
      - Other components
    - (iv) Vitamin C
    - (v) Vitamin D
    - (vi) Vitamin E
    - (vii) Vitamin K
  - (*c*) Other factors:
    - (i) Lactose
    - (ii) Soft curd milk
    - (iii) Minerals
    - (iv) Milk proteins
    - (v) Milk fat
- B. Treated milk
  - (*a*) Heated milk:
    - (i) General
    - (ii) Vitamins
  - (*b*) Irradiated and vitaminized milk
- C. Milk products
  - (*a*) Cheese and whey:
    - (i) Cheese
    - (ii) Whey
  - (*b*) Dried and evaporated milk:
    - (i) General
    - (ii) Vitamin $B_1$
    - (iii) Riboflavin
    - (iv) Nicotinic acid and other members of the vitamin $B_2$ complex
    - (v) Vitamin C
    - (vi) Other factors

IV. Nutritional value of milk for human beings
- A. General
  - (*a*) Introduction
  - (*b*) Nutritional surveys
  - (*c*) General effects
- B. Milk components
  - (*a*) Vitamins
  - (*b*) Proteins
  - (*c*) Minerals
- C. Effect of treatment
  - (*a*) Effect of heating and processing
  - (*b*) Irradiated and vitaminized milk
  - (*c*) Soft curd milk


## I. INTRODUCTION

This review covers a longer period than the preceding ones, from the beginning of 1940 to the end of 1942. War conditions have made access to some sources of information more difficult. However, the appearance of *Dairy Science Abstracts* has greatly facilitated the task of collecting the bibliography. The reviewer wishes to put on record his indebtedness to Mr W. G. Sutton, Deputy Director of the Imperial Bureau of Dairy Science, to Miss D. Knight, Librarian of the National Institute for Research in Dairying, and to their staffs for helping him in every possible way. The scope of the review has remained unaltered, and as usual the papers which have not been consulted in original are marked with an asterisk in the bibliography.

## II. GENERAL

*Food and Life*, the yearbook of the U.S. Department of Agriculture (1) for 1939, deals comprehensively and authoritatively with many aspects of the nutritive value of milk and milk products. Chapters on the nutritive value of milk will be found in the book on *The Market Milk Industry* by Roadhouse & Henderson (2) and in the second edition of Davies's *The Chemistry of Milk* (3). The original analytical tables of McCance & Widdowson (4), the extensive compilation by Chatfield

& Adams (5) and that by Bowes & Church (6) contain much information on dairy products. Kon (7) has compared the relative nutritive value of different forms of milk. A discussion of the economical value of milk (butter) and cheese as sources of protein, calcium, iron and vitamins A, $B_1$, C and riboflavin will be found in (8).

## III. NUTRITIONAL VALUE AS ESTIMATED IN LABORATORY AND FIELD EXPERIMENTS

### A. Raw milk (and butter in the cases of vitamins A and D)

#### (*a*) *General*

Anderson *et al.* (9) found that whole raw milk supplemented with iron, copper, manganese and cod-liver oil allowed good growth and reproduction in dogs, while Evans & Phillips (10) maintained five generations of rats on a diet of mineralized milk.

Investigations on the 'grass-juice factor' have been continued (11,12). According to Johnson *et al.* (12), who used guinea-pigs, the quantity of the factor in winter milk may be increased by feeding phosphoric acid silages to cows; other silages were less effective. In New Mexico, in a study of the deficiencies of a diet of cotton-seed meal and hegari fodder, one group of goats and one of cows received this diet alone, two other groups had access to Sudan grass pasture. When the mineralized milk (4 % fat) was given to rats no evidence was obtained that lack of grass-juice factor might be one of the deficiencies of the ration (13), presumably because rats receiving both types of milk grew alike.

According to Gomez *et al.* (14) milk or oat-juice extract accelerate, when added to an experimental ration, vaginal opening and stimulate early ovarian activity in rats.

Hartman & Cary (15) have found that the subnormal growth of rats on synthetic diets containing yeast or supplemented with various pure vitamins can be improved by the addition of fresh milk, cheese or dried skim milk. The factor (or factors) is stable to heat at *p*H 3–11.

Rupel *et al.* (16) found that the addition of urea as protein substitute to a poor ration of maize silage, timothy hay and a grain mixture, increased the fat, protein and vitamin C content of the milk.

Valenzuela (17) immersed strips of copper or brass in milk for from 5 to 24 hr. and then gave such milk or ordinary milk to rats as an exclusive diet for 8 weeks. There was no difference in the growth rate and no toxic symptoms were observed. Abbott (18) discusses in a general article the nutritive value of skim milk; Bunger (19) advocates curdled skim milk for pig feeding.

#### (*b*) *Vitamins*

(i) *General*

Revised tables of the vitamin content of foods, giving comprehensive figures for the vitamin A, carotene, vitamin $B_1$, riboflavin, vitamin C and vitamin D content of milk and milk products have been published by Fixsen & Roscoe (20), and an equally ambitious survey covering similar ground comes from America (21). An excellent article on vitamins in milk will be found in (1).

(ii) *Vitamin A and carotene*

A brief general review of methods for the assay of vitamin A in butter appears in the *Journal of Milk Technology* (22). Several papers deal with modifications and improvements in the chemical and physical determination of vitamin A and carotene in milk. Most valuable information on the spectrophotometry of vitamin A and carotenoids and on their estimation in milk will be found in the new edition of Morton's book (23). Full technical details and instructions are given in a paper by the same authority (24). In the United States, Neal *et al.* (25) have also given a detailed description of a modification of Gillam's spectrophotometric method (26). They carry out a 'blank' measurement on a portion of the solution of the unsaponifiable matter of butter in which vitamin A and carotenoids have been destroyed by ultra-violet irradiation. Photoelectric methods are rapidly gaining in popularity; Koehn (27,28) has described their application to the measurement of vitamin A and carotene in milk, and Shrewsbury *et al.* (29) to the estimation of carotene in butter fat. A more elaborate photoelectric spectrophotometer for these purposes has been described by

Thompson(30). Simple rapid methods for the measurement of the yellow colour of milk by means of a 'lactochrometer'(31) and of carotene in butter fat by visual comparison with potassium bichromate standards(32) have been described. Effern(33) has applied the reaction of Rosenthal & Erdély(34) to the photometric estimation of vitamin A in butter, but the value of this method is not proven and the calculations are open to criticism.

In previous reviews(35,36,37) papers dealing with the apparently abnormal behaviour of vitamin A and carotene in butter were discussed, but evidence is now growing that the vitamin A activity of milk or butter calculated from proper chemical and physical measurements of vitamin A and carotene agrees with results of biological assay(25,38). The recent results of Fraps *et al.*(39) correcting their earlier findings also point in the same direction, while Shrewsbury & Kraybill(40) now report that carotene dissolved in butter fat devoid of vitamin A activity has the same availability for rats as when dissolved in cotton-seed or maize oil. On the other hand, Koehn(27,28) is still of the opinion that the factor for converting colorimetric readings into international units of vitamin A is appreciably higher for milk than for cod-liver oil.

Normally the fat-soluble pigment of milk consists mostly of $\beta$ carotene and the ratio of 94 % $\beta$ carotene and 6 % xanthophylls put forward by Gillam(41) has been generally accepted. It has been known, however, for some time that the exact proportions vary according to the composition of the carotenoids in the fodder of the cow(37). More recent work in which the analysis was done by chromatography instead of by phase separation indicates that even in normal milk the contribution of $\beta$ carotene may be smaller than hitherto believed, while special feeding of the cow may result in the appearance in the milk of much inactive carotenoid. Thus Johnson *et al.*(42,43) found in the butter of cows receiving molasses lucerne silage only 68 % of the total carotenoids in the form of $\beta$ carotene, the rest consisting of xanthophylls, 24 %, and 'acid-formed' pigments(44), 8 %. Phosphoric acid lucerne silage yielded 65 % $\beta$ carotene and a higher proportion, 12 %, of pigments formed by the action of acid. Even pasture butter had 20 % of xanthophyllic pigments, but artefacts were absent and carotene amounted to 80 %(42,43). Similar values for pasture butters are reported by Thompson *et al.*(45) who also observed that Guernsey cows are less selective than Shorthorns and pass into their milk under comparable conditions a larger proportion of inactive carotenoids. This is doubtless another expression of the known peculiarities of carotenoid metabolism of the Channel Island cattle. There are further examples of these peculiarities. Thus, Gillam & Kon(46) showed that the Shorthorn cow effectively excludes lycopene, an isomer of carotene, from her milk, even when large quantities of it are fed in the form of tomato purée. Under similar conditions Thompson *et al.*(45) isolated and identified lycopene, which, at its highest concentration, amounted to some 12 % of the total pigments of the milk of a Guernsey cow.

The characteristic yellow colour of Guernsey and Jersey milk, apart from being a manifestation of the relative inefficiency of these breeds in converting carotene into vitamin A, is also, commercially, a good selling point. Several papers published in the United States(47,48,49,50) deal with seasonal variations in the colour, genetic factors affecting its inheritance, its correlation with other milk constituents and, finally, with methods for maintaining a high colour throughout the year. In all these studies the 'lactochrometer'(31) was used.

The effect of various fats, including butter fat, on the utilization of carotene by rats was studied by Sherman(51).

Further work by Hauge *et al.*(52) on the vitamin A depressing factor in soya beans has shown that it can be removed from soya bean oil by an activated carbon ('Nuchar'), and that the feeding of such treated carbon to cows causes a lowering of the vitamin A content of the milk; untreated 'Nuchar' was without effect. As in previous experiments(37), the depressing factor did not affect the carotene content of the milk. On the other hand, Williams(53) reports that rations containing soya-bean oil or soya beans (50–78 % of the grain ration) in addition to maize silage and lucerne or timothy hay produced much paler butter fat. Tauber(54) is of the opinion that an unsaturated fat oxidase is responsible for the depressing effect of soya beans on the vitamin A of milk; one would, however, expect this enzyme to act by destroying carotene, which does not tally with the findings of Hauge *et al.*(52).

Observations on fluctuations in the vitamin A and carotene content of milk have again come from several countries. In the United States Dornbush *et al.*(55) have carried out in the course of

two years a careful study of these factors in ordinary market milk, in milk of enhanced vitamin D potency, in Guernsey milk and in certified milk, all of them from Wisconsin farms. The vitamins were measured spectrophotometrically. The total vitamin A activity (vitamin A was measured at 328m$\mu$. using the 1600 basis, 0·3$\mu$g. being taken as equivalent to 1 i.u.) per g. of fat was very similar for all types of milk and amounted for the pasture feeding period from June to October to over 50 i.u./g. and for winter milk (January-April) to about 30 i.u./g. Owing to its higher fat content the Guernsey milk had, on the fluid basis, a higher potency than the other milks. Neal *et al.*(25), who studied the vitamin A activity of American dairy butter for over a year, reported values ranging from 13 to 56 i.u./g., while Booher & Marsh(56) found in biological experiments that summer butter from one source had 59 i.u./g. and two kinds of winter butter, 30 and 36 i.u./g. (The equivalent figures for butter fat would be 72, 37 and 44 respectively.) Summer cream (20 % fat) contained 16 i.u./ml., winter milk 0·87 and summer milk 2·3 i.u./ml. The value of 0·17 i.u./ml. for skim milk containing about 0·02 % fat appears unduly high. In Norway, Breirem(57) measured spectrophotometrically the vitamin A potency of milk produced under different conditions of feeding. For winter milk (roots and hay) he obtained values of 27–31, for pasture milk 60, and for milk produced on A.I.V. silage, root tops and kale, 43–50 i.u./g. fat. Hutchinson(58), in Australia, examined for vitamin A in the course of twelve months 168 samples of mixed milk of over 6000 cows from New South Wales, Victoria and Tasmania. Carotene was measured colorimetrically and vitamin A with the Hilger Vitameter. The mean value was 10·4$\mu$g. carotene and 47·6 i.u. vitamin A/g. fat, or a total vitamin A activity of over 60 i.u./g. No seasonal fluctuations are apparent in the individual values quoted. Paper(59) shows, however, that, under drought conditions, the colour of Australian butter may be appreciably lowered. Seasonal and geographic variations in the colour of New Zealand butters have been reported(60). Values for milk from stall- and pasture-fed cows in Sweden are given in (61); a sample of butter examined in March contained 28 i.u./g. of vitamin A and 1·4$\mu$g. of carotene. Guggenheim(62) has followed seasonal changes in the vitamin A and carotene content of Palestinian milks. His figures for carotene are within the usual limits, but those for vitamin A are strikingly low, from 3·8 to 15 i.u./g.; moreover, the ratio carotene:vitamin A fluctuates markedly and is higher in summer than in winter. This is at variance with general experience. The analytical methods are not described. Papers from India deal with the vitamin A content of milk(63) and of ghee(64,65); for the latter the values quoted in both papers are about 10$\mu$g./g., though in paper(64) calculations are on the basis 1$\mu$g. vitamin A = 3 i.u. and in (65) on the basis 1$\mu$g. vitamin A = 1·56 i.u. Exposure of ghee to sunlight in Petri dishes resulted in the loss of one-third of the vitamin A in 10 min. and in complete destruction within half an hour(64); Doctor & Banerjee(66) found, however, complete loss within 10 min. from layers of similar thickness. Govindarajan & Banerjee(67) report that vitamin A in ghee lost during storage or through exposure to heat and air is regenerated on treatment with hydrogen under pressure. The claim is based on changes in the intensity of the $SbCl_3$ reaction, measured colorimetrically, which may have been caused by removal of inhibitors. It will be hardly accepted without biological proof. Ahmad(68) has published a compilation of the vitamin A content of Indian dairy products. In this country Morton *et al.*(24) found values of 29–39 i.u./g. fat for the milk of pure-bred Ayrshires grazing between August and October, while for the bulk milk of the Shinfield herd Henry *et al.*(38) observed potencies varying from 23 i.u./g. fat in March to 48 i.u./g. fat in August. A discussion of the vitamin A content of summer butter will be found in paper(69).

A number of papers have dealt with the effect of various fodders on the colour and on the carotene and vitamin A content of milk. It is the general opinion that if fed in sufficient quantity, properly made lucerne and grass silages produce milk which is little, if at all, inferior in colour to pasture milk(70,71). The findings of Johnson *et al.*(42,43) should, however, be borne in mind. The carotene content of the silages used in (71) is given in (72). Maize silage gave less satisfactory results(70). Johnson *et al.*(73), by feeding phosphoric acid and molasses lucerne silage, produced milks of potencies up to 50 i.u./g. fat and well above the average for the market milks for the same time of year(55). Dehydrated sweet potato contains appreciable amounts of carotene (some 100$\mu$g./g.), and tests in South Carolina have shown that this is well utilized by cows(74). The feeding of pea and vine silage was also found useful(75). On the other hand, sorghum products(76) and ordinary potato(77) have an unfavourable effect on the carotene content of butter.

By feeding shark-liver oil, a potent source of vitamin A, to cows, Deuel *et al.*(78,79,80,81) produced milk very rich in vitamin A. Thus 4,200,000 i.u. per cow daily induced in the butter of a group of Holstein cows a potency of 280 i.u./g. fat. The highest individual value was 331 i.u./g. For Guernsey cows the potencies were slightly lower. The increase in the potency of butter was proportional to the quantity of vitamin A fed. The efficiency of transfer was about 3 %. It is of interest that the carotene content of the blood and milk of the cows was decreased by feeding shark liver oil, an effect of vitamin A *per se*, rather than of the oil. These findings have since been confirmed by Blaxter *et al.*(82), who fed shark-liver oil to Shorthorn cows, and by giving 2,400,000 and then 4,800,000 i.u. daily produced butter of a maximum potency of 268 and 390 i.u./g. fat respectively. The carotene content was again depressed.

A similar depressing effect of vitamin A was observed by Martin *et al.*(83). It is surprising that Rusoff *et al.*(84), who gave cows from 200,000 to over 2,000,000 i.u. daily of vitamin A as shark-liver oil in addition to some 750,000 i.u. derived from the basal diet, produced no increase in the vitamin A potency of the milk. The oil was mixed with the concentrate ration, and it is not clear that stability tests were done.

It is well known that the vitamin A and carotene content of milk cannot be raised indefinitely by increasing the carotene content of the fodder and that 'ceiling' values are reached at about the levels found in pasture milk(36). Thus Wilbur *et al.*(85) reached saturation point with about 550,000 i.u. daily of carotene as artificially dried lucerne. The milk had a potency of 31 Sherman Munsell units/g. fat (approximately 45 i.u., as one S.M. unit is said to be equivalent to 1·4 i.u.(86)). An increase of the carotene intake to 850,000 i.u. did not raise the potency of the milk any further. 140,000–280,000 i.u. daily of vitamin A itself produced the same effect as 550,000 i.u. as carotene(87) but it is not clear whether the authors claim that a 'ceiling' for vitamin A feeding was also reached. This seems most unlikely in view of the findings of Deuel *et al.*(78,79,80,81) and Blaxter *et al.*(82).

Deuel *et al.*(78,80) have also claimed that the feeding of shark-liver oil increases the milk and fat yield of cows. This has not been confirmed either by Rupel *et al.*(88) or by Blaxter *et al.*(82), but Wilson(89) has recently reported similar observations.

Hilton *et al.*(90) found by biological assays on the milk that there was no difference in the availability for cows of the carotene in lucerne hay or when dissolved in oil. The work of Copeland & Fraps(91) would indicate, however, a better availability from the natural source.

The question of oxidized flavour in milk has continued to receive much attention in the United States where the fault is of definite practical importance. A very comprehensive review dealing with all aspects of this complicated problem has been published by Brown & Thurston(92). It shows that the consensus of opinion is that carotene is a factor tending to reduce the susceptibility of milk to oxidation, and this view is supported by Garrett *et al.*(70) and Martin *et al.*(83), who also found carotene effective in reducing the intensity of copper-induced flavour, though Brown *et al.*(93) are of the opinion that it is of little value in this connexion and that the reduced susceptibility of milk following the feeding of lucerne hay and meal is caused by some other substance. Williams & Burgwald(94) found that added carotene exerted no inhibitory effect on spontaneous oxidized flavour in milk.

Miller *et al.*(95) have found that in mastitis, artificially induced by injection of *Str. zooepidemicus*, the vitamin A and carotene content of the milk from the infected quarters was more than doubled. Kon & Thompson(96) observed the same interesting phenomenon in naturally occurring mastitis. This is the more striking as at the same time the concentration of the water-soluble vitamins was definitely reduced.

Richter(97) has studied the change in concentration of carotene in successive samples of colostrum of four black and white cows taken three times daily for the first 10 days after calving, and Phillips *et al.*(98) have demonstrated the marked effect of the ingestion of colostrum on the blood vitamin A level of the new-born calf, thus confirming the earlier findings of Dann(26). Stewart & McCallum(99) found that neither the feeding to cows of 3 lb. of carrots nor of one-seventh of a pint of cod-liver oil daily, in addition to low-carotene rations, for 2–5 months before calving, increased the vitamin A and carotene content of their colostrum over that of control cows. They believe that the level of vitamin A in colostrum is independent of the body reserves of vitamin A. It should be remembered, however, that the quantities they fed were relatively

small, 70,000 i.u. daily of vitamin A and probably not more than 150,000 i.u. daily of carotene, and it would be of interest to know whether higher doses would produce an effect.

Reports on a long series of experiments dealing with the effects of various fats, treated and untreated, on the vitamin A activity of butter will be found in (100,101), while a method of protecting vitamin A in butter and dairy products by ascorbic acid and related compounds is described in (102).

Krukovsky(103) found that when milk fat was subjected to fractional crystallization the concentration of carotene in the different fractions followed the same trend as the iodine number and was inversely related to the melting-point.

A general article on margarine has appeared in (104), and an authoritative and detailed description of the spectrophotometric assay of vitamin A in margarine is given in (105).

(iii) *The vitamin B complex*

The list of identified factors of the vitamin B complex has increased considerably, and there is evidence that one at least of the more recent additions is required by man(106).

Chemical, microbiological or biological methods of assay are available for most of these factors and much work has been published on their presence in milk. Various improvements in existing methods have also been reported. An estimate of the content of various members of the vitamin B complex in milk is given by Williams(107). A recalculation of his figures on the assumption that they are per 500 Cal. and that 100 ml. of milk yield 70 Cal. gives the following values in $\mu$g./100 ml.: vitamin $B_1$ 43, riboflavin 200, nicotinic acid 57,* pantothenic acid 310, biotin 8·6, inositol 14,000, vitamin $B_6$ 30, 'folic acid' 15. Similar estimates and a description of the methods used will be found in an important monograph from Williams's laboratory(108).

*Vitamin* $B_1$. Modifications and improvements of the thiochrome test and their application to the assay of milk and milk products are described in (109, 110, 111, 112, 113, 114). In all these the precautions necessary to determine the total (free and bound) aneurin (cf. (37)) were taken, and the procedures described in (109, 111) were checked by biological assay. Values for raw milk fluctuate between 9 and 16 i.u./100 ml. Bertagni(115), who apparently measured only the free form, reports lower figures.

Other techniques applied to dairy products were the diazo method of Prebluda & McCollum(116) used by Halliday & Deuel(117) and by Sakurai(118), the *Phycomyces blakesleeanus* method (cf. (36)) applied by Hutchinson(58), the yeast (*Saccharomyces cerevisiae*) growth method of Williams *et al.*(119) and the yeast fermentation method of Schultz *et al.*(120). Finally, results of biological tests on rats are reported by Booher & Hartzler(121), Knott(122) and Morgan & Haynes(123). The first quote values of 14 and 16 i.u./100 ml. for skim and whole liquid milk respectively, the last give 9–11 i.u./100 ml. for whole milk. Hutchinson's(58) mean figure for the large series of Australian milks (cf. p. 219) was 18·8 i.u./100 ml.

Halliday & Deuel(117) have studied the vitamin $B_1$-protein complex of milk.

*Riboflavin.* A good review of the factors controlling the riboflavin content of milk and milk products will be found in (124). A method for the preparation of pure riboflavin concentrates by selective adsorption on lactose crystallizing from concentrated whey, is given by Leviton(125). Modifications of fluorimetric methods for the assay of riboflavin applicable to milk and its derivatives have been described by Chapman & McFarlane(126), Conner & Straub(110) and Najjar(127). The last procedure has given excellent results in the laboratory of the reviewer. Two reports have been published (128,129), giving the results of collaborative comparisons of fluorimetric, bacteriological and colorimetric methods for the assay of riboflavin in milk. While the first two methods were in good agreement the last proved less reliable. Rat assay methods have been described and applied to milk products by Wagner *et al.*(130) and Munsell(131). The latter paper contains, in addition to original figures, a compilation of data from the literature. Values for the riboflavin content of milk have been reported from Holland(132), Sweden(61), the Dutch East Indies(133), Japan(134), Canada(126) and the U.S.A.(131).

The findings of Johnson *et al.*(135) support the evidence produced in the previous review(37) that pasture milk is richer in riboflavin than that of stall-fed cows. They also confirm that the effect of diet on the riboflavin content of milk is only limited, and that the relationship between

* This value is lower than that quoted in (108).

the concentration of the factor in fodder and milk is far from clear-cut. The explanation most probably lies in the fact that the cow obtains riboflavin not only from the food but also by endogenous synthesis (135, 136, 137, 138, 139), and, indeed, it has been shown by McElroy & Goss (136) that a cow receiving a diet almost free from riboflavin, produced milk containing normal concentrations of the factor. The work of Johnson *et al.* (135) supplies further proof that riboflavin is not related in the cow to the synthesis of milk fat (cf. (37)). It shows, however, that the concentration of the factor in milk is inversely related to milk yield.

It would appear that the mechanism controlling the passage of riboflavin into the milk differs from that dealing with vitamin $B_1$: the latter factor is also synthesized in the rumen (137, 138, 139, 140), but cannot be affected by dietary means (cf. (26, 37)).

The flavoprotein of milk (cf. (37)) is associated with the fat surface, passes into raw cream, and probably accounts for the reddish colour of raw cream buttermilk (124). This explains the finding that dried commercial buttermilk is twice as rich in riboflavin as dried skim milk (cf. (37)). The compound progressively decomposes on heating and complete liberation of flavin takes place at 80° C. Hence skim milk from pasteurized milk is richer in riboflavin than that from raw milk. The reverse is naturally true of buttermilk (124, 141).

*Nicotinic acid.* Methods specifically designed for the chemical determination of nicotinic acid in milk have been described by Bailey *et al.* (142) and Noll & Jensen (143), while the application to dairy products of more general methods has been reported by several authors. Papers (144, 145, 146, 147, 148, 149, 150) deal with chemical and papers (151, 152, 153) with microbiological technique. Finally, Waisman *et al.* (154) and Schaefer *et al.* (155) have published results of biological assays on dogs. Compilations of data from the literature will be found in (156, 157). The findings are rather variable, low values around 80$\mu$g./100 ml. are given by the microbiological assay with *Lactobacillus arabinosus*, the chemical methods yield figures lying between 60 and 500$\mu$g./100 ml., while biological assays indicate a content of 400–500$\mu$g./100 ml. It is of interest that the methods specifically designed for milk give values of the same order as those of the microbiological assay.

Wegner *et al.* (138, 139) have demonstrated synthesis of nicotinic acid in the rumen.

*Pantothenic acid.* Pelczar & Potter (158) used *Proteus morganii* and Pennington *et al.* (159) and Bauernfeind *et al.* (160) *Lactobacillus casei* $\epsilon$ for the microbiological assay of dairy products. Strong *et al.* (161) obtained by a similar method a value of 4–7$\mu$g./g. of fresh milk. McElroy & Goss (162) showed, together with (138, 139), that pantothenic acid is synthesized in the rumen and found on the basis of a chick assay a value of 46$\mu$g./g. for the dried skim milk of a cow receiving a diet deficient in the factor. The two values are thus in excellent agreement and agree also with Williams's (107) figure. Jukes (163), using chicks, obtained values for skim milk, whole milk and whey of 2·1–4·3, 1·3–4·2 and 2·4–5·7$\mu$g./ml. respectively. For buttermilk the figures were 3·5–5·6$\mu$g./g.

*Vitamin* $B_6$. Using a chemical method Swaminathan (164, 165) found 1·7$\mu$g./g. milk in very good agreement with the value of 1·3$\mu$g./ml. obtained by Henderson *et al.* (166) by a rat growth method. With rats, on the other hand, Henry & Kon (167) found lower values of 3–4$\mu$g./g. for dry skim milk. These agree, however, with the estimate of Williams (107, 108) using a microbiological method, but are higher than the value of 0·17$\mu$g./ml. reported by McElroy & Goss (168) from biological assays of normal milk and of milk produced by a cow on a deficient ration. Here again synthesis of the factor in the rumen has been proved (138, 139, 168).

*Biotin.* Using different microbiological methods Lampen *et al.* (169), Nielsen & Hartelius (170), and Shull *et al.* (171), obtained values of 3–4, 0·7–2·6 and 1·2$\mu$g./100 ml. respectively. Williams's figure (107, 108) is given on p. 221. Melville *et al.* (172) have isolated crystalline biotin from milk concentrates. Synthesis of the factor in the rumen has been established (138, 139).

*Inositol.* Woolley (173) found in whole milk 0·5$\mu$g./mg. solids equivalent to some 6 mg./100 ml. This is about half Williams's (107, 108) figure (cf. p. 221).

*Other components.* Skim milk contains about 0·4 mg./g. of choline hydrochloride (174). The presence in liquid milk of a labile substance having the same curative effects in gizzard erosion in chicks as cholic acid has been reported. Cholic acid itself could not be demonstrated in milk (175).

(iv) *Vitamin C*

A compilation of data on the vitamin C content of milk is given by Guigoz (176). A useful bibliography will be found in (177). Gunsalus & Hand (178) have described a method for the micro-

biological reduction of dehydroascorbic acid by means of suitable strains of *B. coli*. The procedure appears much simpler than the usual treatment with $H_2S$, and, if its value is confirmed, should prove most useful in the determination of total ascorbic acid in milk. Woessner *et al.*(179) have given details of the photoelectric method previously described(37). Holtz(180) claims that a bound form of vitamin C is present in milk and that this must be decomposed before titration, by heating to 100° C. in the presence of HCl. Wachholder & Okrent(181) deny the existence of the compound and ascribe Holtz's findings to faulty technique.

Measurements of vitamin C in commercial milk supplies are reported from Australia(58), Palestine(62), India(63), Sweden(61), the Argentine(182), Finland(183), Norway(57) and the United States(184). The first two deal only with the reduced form.

Holmes *et al.* have studied the effect of the age of the cow, stage of lactation, length of lactation period, pregnancy, and yield on the concentration of vitamin C in milk(177,185,186,187,188). On the whole, none of these factors exerted any marked effects and the vitamin C content fluctuated around the usual figure of about 2 mg./100 ml. It follows that the total daily output of vitamin C was roughly proportional to the milk yield. Work by Wallis(189,190) gives further proof that the vitamin C content of milk and blood plasma is independent of that of the diet, values for cows receiving a deficient ration being as high as for cows on pasture. Balance experiments with the former(190) showed that in one instance on a daily intake of 73 mg., 526 mg. were secreted in the milk and 703 and 19 mg. excreted in faeces and urine respectively. The faecal ascorbic acid could not be derived from the feed, and indeed Knight *et al.*(191,192) have shown that ingested ascorbic acid is rapidly destroyed in the rumen. When large quantities of the vitamin were injected intravenously there was a slight but definite rise in the level in the milk, but the bulk was excreted in the urine(192).

There is thus little doubt that the cow synthesizes vitamin C in her body, but the various members of the vitamin B complex are most probably derived from bacterial symbiosis. The findings of Ray *et al.*(63) point in the same direction.

Though remarkably stable under normal conditions, the vitamin C level of milk may vary appreciably in special circumstances. As mentioned in the previous review(37), a marked rise follows the administration of cod-liver oil. More recent work(193,124) has shown, however, that this is a nervous and not a dietary phenomenon; it can be elicited by drenching with but not by ordinary feeding of the oil. Drenching with 5 g. daily of potassium iodide produced the reverse effect. There was a decrease of about one-quarter to one-third in the ascorbic acid level which promptly returned to normal when the treatment was discontinued(194). It was found some time ago(195,196,37) that in mastitis the vitamin C level is low, and this finding has now been abundantly confirmed(197,96). Sharp and his collaborators have continued their work on the deaeration of milk, a procedure which protects the vitamin C from effects of exposure to light, heat and copper and prevents the development of oxidized flavours(198,199,200,184), and Noll & Supplee(201) have carried out similar studies. Sharp *et al.*(202) have also published an ingenious method for the quantitative determination of dissolved oxygen in milk and other fluids based on the oxidation of ascorbic acid in the presence of ascorbic acid oxidase from cucumber.

Much work has been published in America on the relation of vitamin C to the development of oxidized flavour in milk(93,194,203,204,205,206,207), and more general reviews of the subject will be found in papers(92,208,209). In general it was found that the addition of ascorbic acid to milk prevented or lessened the development of oxidized flavour(204,205,206), though Corbett & Tracey(203) express doubts as to the efficacy of this procedure. It is likely that ascorbic acid, owing to its low $E_h$, inhibits the oxidation of some minor constituent of milk which gives rise to the flavour. The amount naturally present in milk is, however, insufficient to be of value, and variations within these normal limits are without effect on the development of flavour(93,194,207).

Reif(210) has studied the effect of various metals and their salts on the vitamin C of milk. As would be expected, Cu was the most deleterious, and the activity decreased in the order Fe, Mn, Ni, Zn and Sn. Ti, Mg and Al were without effect and chlorides and sulphates of Sn, Zn and Ni were slightly protective. Garrett(211) investigated the action of Fe, Mn and Al on the development of copper-induced oxidized flavour.

Dills & Nelson(212) isolated from milk a copper-bearing protein which, rather surprisingly, did not exhibit any ascorbic acid oxidase activity. Noll & Supplee(201) confirmed that the reversible

photo-oxidation of vitamin C is a monomolecular reaction. Henderson *et al.*(213) compared the value of three types of cartons in general use in the United States in protecting the vitamin C and flavour of milk exposed to sunlight. All were better than clear glass, and those with unbleached inner plies superior to the rest. The effect of light on ascorbic acid proved a good index of the effect on flavour. This subject was also studied by Woessner *et al.*(179). According to them the loss due to light takes place mainly after delivery to the consumer. England & Wiedemer(214) and Roadhouse & Henderson(215) studied the development of spontaneous and copper-induced oxidized flavour in milk exposed to sunlight. While cartons gave more protection than clear glass bottles, the treatment of the cartons with antioxidants (oat flour) did not increase the degree of protection(213).

(v) *Vitamin D*

Much useful information and an exhaustive bibliography will be found in Weckel's(216) authoritative review on vitamin D in milk. Relatively few original papers dealing with this vitamin have appeared. Gridgeman *et al.*(217) compared the line test and the bone-ash test in the estimation of the vitamin D content of margarines and found that when the assay was done against the international standard for vitamin D (irradiated ergosterol) the estimate of potency by the bone-ash method was some 10 % lower than by the ash test. The discrepancy was not observed when pure calciferol was used as standard. Henry & Kon(218) have studied the vitamin D content of English butter fat at monthly intervals throughout the year. To avoid the 'fat effect'(37) the assays were carried out on the unsaponifiable residue of the fat. Between November and March the potency was at an almost constant low level of 0·07–0·1 i.u./g. The highest value was 0·97 i.u./g. at the end of August (owing to extrapolation in calculating, this value may have been too high), the next highest value was 0·55 i.u./g. at the beginning of July. At the beginning of August and at the end of September the potency was 0·38 and 0·35 i.u./g. respectively. On four occasions simultaneous assays were done on the fat itself, and it was found that, as would be expected, the 'fat effect' was more marked in winter than in summer; winter butter lost on saponification some three-quarters of its potency, summer butter only one-third.

A report from Arizona(219) confirms the findings described in earlier reviews(36,37) that the summer increase in the vitamin D content of milk is due to the direct action of the sun on the cow.

Dogs receiving mineralized milk as the sole diet developed mild vitamin D deficiency characterized by low blood phosphorus. An increase could be produced by the administration of cod-liver oil(220).

Booth *et al.*(221) made a detailed study of the 'fat effect'; they found that vegetable fats devoid of vitamin D and triglycerides resynthesized from the fatty acids of butter after careful removal of the non-saponifiable residue exerted a measurable antirachitic effect on rats receiving high Ca-low P rachitogenic diets. A metabolic experiment showed that the addition of fat lowered the faecal excretion of P and the urinary excretion of Ca. Fat had, however, no calcifying effect when added to high P-low Ca diets.

(vi) *Vitamin E*

According to Anderson *et al.*(9), whole raw milk as an exclusive diet (supplemented with minerals and cod-liver oil) supplies enough vitamin E to support good growth and reproduction in dogs. An earlier paper by the same authors (222) mentions vitamin E deficiency in dogs fed on raw milk. Mason & Bryan(223) report that milk is low in vitamin E and that butter from cows on pasture must be incorporated into vitamin E-deficient diets to the extent of 20 % to confer fertility on rats receiving such diets from weaning.

(vii) *Vitamin K*

Salomonsen(224) found that the administration of from 30 to 60 ml. of cow's milk to new-born infants prevented the usual fall in prothrombin and thought that the milk provided a culture medium for intestinal bacteria which then produced vitamin K. Sells *et al.*(225) are, however, of the opinion that the infant's requirement for vitamin K is low and that the factor was directly supplied by the milk. Buruiana(226) has prepared a fraction from milk which exhibited vitamin K activity, and McElroy & Goss(136) have demonstrated the synthesis of this factor in the rumen.

### (c) *Other factors*

(i) *Lactose*

Roberts & Christman(227) have briefly reviewed the literature dealing with the beneficial effects of lactose on the assimilation of calcium. Their own experiments on rats by the method of Cori showed that neither lactose nor galactose increased the rate of absorption of calcium lactate from the gut. Other calcium salts were not tried. Using the same technique Leonards *et al.*(228) found that the uptake of galactose was slowed down in vitamin $B_1$ deficiency. The utilization of lactose by the rat has been studied by Nalder(229). Richter(230) offered various carbohydrates as the sole diet to rats and found that their appetite was least for, and the survival time shortest with, lactose and galactose. Mitchell *et al.* have continued their studies of the mechanism of development of lactose (galactose) cataract(231, 232, 233, 234). They confirmed the protective action of protein, an effect also noted by Yudkin & Geer(235). The amino acid or acids exerting this action were apparently different from those essential for growth. Urea and cystine were slightly protective and histidine also proved effective. Deaminized casein fed with casein proved more effective than casein itself. The lenticular changes produced by galactose in rats are said to be similar to those in senile human cataract(236). Selle(237) has claimed that galactose given subcutaneously failed to produce cataract, but he gave amounts equivalent to not more than 10 % of galactose in the diet, a concentration insufficient, according to Yudkin & Geer(235), to cause definite changes even when fed for long periods of time. The latter authors have shown, moreover, that galactose injected intraperitoneally is effective. According to Darby & Day(238) xylose is also cataractogenic, but to a less extent than galactose. They ascribe this property to similarity in chemical configuration of the two sugars. Patch(239) observed cataracts in Salamander larvae receiving diets containing 45 % purified casein and 37 % dried whole milk.

(ii) *Soft-curd milk*

Dizikes & Doan(240) have described a new type of soft-curd milk prepared by a method similar to that commercially used in making superheated evaporated milk. A method for the preparation of soft-curd milk by treatment with sodium hexametaphosphate has been described(241). Kelly(242) has studied (*in vitro*?) the digestion of soft-curd (homogenized) milk.

(iii) *Minerals*

Archibald & Parsons(243, 244) have confirmed earlier findings(36) that the iron content of milk cannot be increased by feeding additional iron. Erf(245) showed that when labelled (radioactive) iron was given to cows as iron sulphate some 2 % of it was secreted in the milk in the course of 78 hr. On the strength of this finding he made the surprising and entirely unwarrantable suggestion that if a cow producing 4 l. of milk daily were given an oral dose of 10 g. of iron she might secrete 0·5 % of it in 24 hr. or 12·5 mg./l. It is of interest that the level of manganese, chemically closely related to iron, can be appreciably affected by feeding(246). The fluorine content of milk can also be varied to a limited extent by this means(247). The commercial production of 'superiodized' milk has been reported(248). Information on the availability of milk calcium will be found in Mitchell's monograph(249).

Milk diets have been used for the study of iron and copper and of cobalt in haemoglobin formation in the dog(250, 251, 252, 253) and in the rat(254), for the study of the manganese requirements of rats(255, 256), and for the demonstration that the fluorine requirement of the rat is of a very low order(10). Free(257) has shown that the greying of rats receiving an exclusive diet of milk is due to a mineral deficiency and can be cured by the administration of iron, copper and manganese. According to Daniel & Hewston(258) vanadium is either entirely absent from milk and milk products or its concentration is less than 1–5 parts per million of the ash of these materials.

(iv) *Milk proteins*

A marked supplementary effect of casein on the biological value of maize proteins for young growing pigs has been reported by Macrae *et al.*(259).

Riboflavin is present in commercial casein to the extent of 6$\mu$g./g. according to Culton & Bird(260), while Baars(133) found about half this quantity. Pelczar & Porter(158) found that casein

contained 19$\mu$g./g. of pantothenic acid, and Bauernfeind *et al.*(160) gave a figure of 5·3$\mu$g./g. The biological assay of Jukes(163) indicates, however, that commercial casein contains less than 0·9$\mu$g./g. Cary(261) described the presence, in commercial casein, of an unidentified growth factor for rats distinct from all known vitamins. The factor was absent from casein freed of vitamin A. It may well be identical with Coward's factor(262).

Griffith *et al.*(263,264,265) and Blumberg *et al.*(266) have studied the relation of casein to choline metabolism in the rat and Meeker & Kesten(267,268) to atherosclerosis in the rabbit. Anderson *et al.*(269) and Gortner(270) have demonstrated the marked protective effect of casein on selenium poisoning in rats. Lactalbumin was less effective(270). Enzymic digests of casein proved at least as effective as casein itself in promoting the growth of rats and regeneration of serum protein in dogs(271,272), and gave satisfactory nitrogen retentions in children(273); they inhibited, however, the growth of chicks(274). Richter(230) found that rats survived longer on a pancreatic digest of casein than on other single proteins (cf. p. 225). Deaminized casein given as the sole protein causes anaemia in rats. This is due to lysine deficiency(275) and can be cured by administration of adequate quantities of the amino acid.

Continuing their important studies of factors controlling the span of life of the rat, McCay *et al.*(276) have shown that the proteins contained in dried liver were superior for longevity to combinations of casein and lactalbumin. It should be remembered that the superiority of liver may have been due to the presence in it of essential factors absent from the isolated milk proteins.

(v) *Milk fat*

An authoritative article on the nutritive value of butter is given in (277). Hart and his collaborators have continued their work on the comparative nutritive value of different fats reported in the previous review(37). They blended 4 % of different fats with skim milk and gave the mixture, suitably supplemented with vitamins and mineral salts, to newly weaned rats. Butter fat produced better initial gains in weight than maize oil, cotton-seed oil, coconut oil, soya-bean oil and 'crisco'. After some three weeks the growth rates became similar for all fats. The superiority of the butter fat was traced to a saturated component or components of its fatty acid fraction, and it was also shown that the unsaturated fatty acid fraction of butter could be activated by hydrogenation(278, 279, 280). Using a similar technique but working with calves, Gullickson *et al.*(281, 282) found that butter fat was superior to tallow and lard and, to a greater degree, to coconut, peanut, maize, cotton-seed and soya-bean oils. Harris & Rosenfeld(283) gave to newly weaned rats extracted skim-milk powder mixed with 25 % of olive oil, coconut oil or butter fat and supplemented with minerals and vitamins. Here again, the rats receiving butter grew better than the other animals. However, a reversal of this effect was observed by Harris & Mosher(284) with adolescent rats which grew better on a skim-milk powder-coconut oil diet than on the same diet with butter fat. Boer & Jansen(285,286) described experiments, of which only summaries are available in this country, in which butter, butter-fatty acids, olive oil, salad oil and margarine were added to a synthetic diet to the extent of 10 %. Butter and its fatty acids caused better growth in young rats than the other oils. Euler *et al.*(287) similarly added fats to a synthetic ration. Margarine blended from 15 % soya-bean oil, 70 % coconut oil and 15 % hard fat proved superior to butter in promoting growth of rats. In Richter's(230) experiments with single foods (cf. p. 225) rats survived on unsalted butter longer than on any other fat. It should be pointed out, however, that butter contains some nitrogenous matter.

Hagnell-Zethræus *et al.*(288) noted in extensive metabolic studies that older growing rats assimilated calcium slightly better in the presence of butter than with margarine, but observed no difference for quite young or mature animals or when the diets were poorer in calcium.

Sherman(51) has confirmed the opinion that butter is low in essential unsaturated fatty acids. On the other hand, Schneider *et al.*(289,290) have shown that fresh butter possesses appreciable antiacrodynic properties, but that these are lost when butter becomes rancid.

## B. Treated milk

### (a) *Heated milk*

(i) *General*

Wilson(291) has reviewed in great detail experiments dealing with the effects of pasteurization on the nutritive value of milk and concluded that the results of various experiments on different animals and on children failed to show that pasteurization of milk by the holder method had any significant effect in lowering its total nutritive value for the growing animal. Elvehjem(292) also finds no objection to pasteurized milk on a nutritional basis. Kirsch(293) gave 14-day-old bull calves either raw or flash-pasteurized milk. The mean gains in weight for the two groups of three were 118·5 and 113·4 kg. respectively in the course of 105 days. No individual figures are given, and it is doubtful whether any significance attaches to the difference.

(ii) *Vitamins*

According to Kendall(113) pasteurization destroys from 10 to 20 % of the vitamin $B_1$ of milk. Thus American market milk contained 41$\mu$g./100 ml. raw and 37 after heat treatment; for certified milk values were 48 and 38$\mu$g./100 ml. respectively. Knott(122) reports much lower values, 26$\mu$g./100 ml. for pasteurized milk, but no figures for raw milk.

Munsell(131) found by the rat-growth test 214$\mu$g. of riboflavin in 100 ml. of pasteurized milk.

Again a number of papers have been published dealing with the effect of pasteurization on vitamin C. Woessner *et al.*(179), who made a detailed study of the destruction of reduced and reversibly oxidized ascorbic acid under commercial conditions, report a loss of some 20 % of total ascorbic acid in the holder process. In the absence of copper the conversion of ascorbic to dehydroascorbic acid has a low temperature coefficient, and pasteurization causes little loss of the reduced form. Dehydroascorbic acid, on the other hand, is rapidly destroyed by heat. In the presence of copper the thermal destruction of reduced ascorbic acid is greatly hastened, but the decomposition of the reversibly oxidized form is not further accelerated. Hartman & Garrett(294) advise tests for vitamin C on suitable milk samples from pasteurizing plants as a means of detecting sources of copper contamination. Flash pasteurization causes smaller losses than the holder process, in fact the American authors(182,295) observed no measurable effect of the former and only a 9 % loss in the latter. Milk from which the oxygen has been removed can be pasteurized and distributed without loss(200,201), and this possibility may be contrasted with the actual mean value of 0·29 mg./100 ml. obtained for 1502 samples of commercial bottled pasteurized milk collected in various cities in the State of New York(200). Gjessing & Trout(207) have dealt specifically with the effect of various heat treatments on vitamin C and the development of oxidized flavour in milk, and further information will be found in papers already quoted on p. 223. Lengthy heating or autoclaving of milk leads to the formation of substances giving a spurious titration with the indophenol reagent(296). Fleisch(297) studied the influence of different kitchen utensils on the destruction of vitamin C and found under practical conditions that the loss was least with aluminium.

Anderson *et al.*(9) confirmed their earlier findings(37) that pasteurized milk is equal to raw in promoting growth in dogs. They found, however, that it is less satisfactory for repeated pregnancy and lactation. The muscle dystrophy observed in pups reared by bitches receiving pasteurized milk could be cured or alleviated by vitamin E therapy (cf. also (222)). The pups also developed a haemorrhagic condition which may have been due to vitamin K deficiency.

Muscular dystrophy was also observed by Bahrs & Wulzen(298,299) in rats and guinea-pigs receiving pasteurized milk, but not when raw milk from a different source was given. The milks were supplemented with iron and copper salts (not manganese), 10 % skim-milk powder, orange juice, iodized salt and, sometimes, a biscuit (cracker) of unstated composition. Wrist stiffness was the first symptom of the dystrophy, and this could be cured by the administration of raw cream. The activity was traced to methylvinylketone. The synthetic substance also proved effective. This rather unusual finding will certainly require independent confirmation.

### (*b*) *Irradiated and vitaminized milk*

While original papers dealing with this subject are few, a number of general articles and reviews has been published, several of them written by leading authorities. Thus McCollum (300), dealing with all aspects of the fortification of milk, expresses himself only in favour of the addition of vitamin D. The views of the Council on Foods and Nutrition of the American Medical Association are reported by Bing (301). The Council allows the addition of vitamin D only to milk of all foodstuffs, and this not beyond a potency of 400 U.S.P. units (equivalent by definition and probably nearly equivalent in actual fact to international units) per American quart. The technical aspects of the various methods of addition of vitamin D to milk, by feeding it to cows, directly to the milk in the form of concentrate, or through irradiation, are discussed in detail in the informative articles by Weckel (302,303). Further information will be found in (304,305). New facts about the mechanics of irradiation are given by Beck *et al.* (306). Statistics concerning the manufacture of irradiated milk in Germany have been published (307). A discussion of irradiated milk comes also from France (308). The laboratory control of vitamin D milk is dealt with by Oser (309), and the technique of the biological assay of its vitamin D content is reviewed in two further collaborative reports from American laboratories (310,311). The addition of vitamin D to milk by any of the recognized methods has no effect on its vitamin A content (55,312) nor does irradiation destroy riboflavin (312), though vitamin C may suffer from longer exposure to ultra-violet light (313); such exposures would, however, not obtain in properly carried out commercial irradiation. Vitamin C is not affected by the addition to milk of vitamin D concentrate (179). The utilization by rats of vitamin D added in this way is not affected by wide changes in the curd tension of milk (314). Fortification of milk with vitamin C is a commercial possibility (179), and as a matter of fact forms part of a scheme of fortification of milk with seven vitamins applied by two dairies in the United States (315,316). One (American) pint of their milk retails at 15 cents and is said to contain 6000 i.u. of vitamin A as carotene, 750 i.u. of vitamin $B_1$, 2 mg. of riboflavin, 50 mg. of vitamin C, 10 mg. of nicotinic acid, 5 mg. of calcium pantothenate and 200 U.S.P. units of vitamin D. The last addition is not made by the Californian dairy (315).

## C. Milk products

### (*a*) *Cheese and whey*

(i) *Cheese*

Henry & Kon (317) obtained by the method of Mitchell a biological value of 75·6 for the proteins of Cheddar cheese and demonstrated a marked supplementary relationship between them and those of white bread. No supplementary effect was shown when the two sources of protein were fed to rats separately on alternate days.

A preliminary report has appeared of the work of Houston & Kon on the vitamin content of Cheshire, Cheddar and Stilton cheese made from one bulk of milk (318). The vitamins of cheese have been studied by a number of authors. The following figures are quoted: vitamin A, American Cheddar (made in April, hence presumably from milk low in vitamin A) 12 i.u./g., Swiss 27 i.u./g. (56); vitamin $B_1$, American Cheddar 14 i.u./100 g. (121,319); riboflavin, American Cheddar made from skim milk 400$\mu$g./100 g., Swiss, 172$\mu$g./100 g. (132), full-cream American Cheddar, 545–600$\mu$g./100 g. (131). Relatively high concentrations were also found in the English cheese (318). All these high values confirm that riboflavin in milk is partly bound with the protein (cf. p. 222), as otherwise much more would be removed in the whey. In the preparation of Swiss cheese the curd is heated to a higher temperature, and this would explain the lower riboflavin content of this type of cheese (124,132) as heating causes the decomposition of the flavoprotein (124,141). For biotin in cheese a value of 2·6$\mu$g./100 g. has been quoted (169), and for nicotinic acid figures are quoted in the compilation of McVicar & Berryman (157).

(ii) *Whey*

The utilization of whey in foods is discussed in (320) and paper (321) stresses its importance in pig feeding.

Improvements in the nutritive value of white bread following the addition of whey to flour are described by Henry *et al.*(322).

Randle *et al.*(11) found a small amount of 'grass juice' factor in dried whey. A figure of 1·69μg./g. is quoted for the vitamin $B_1$ content of dried whey(109). The riboflavin content has been studied by (110,126,260,323). In the last paper are given values for 244 samples of dried whey obtained in the course of 2 years from six different factories in five different States of the U.S.A. There was very little seasonal variation (cf. p. 221), the mean value for winter was only 7 % lower than for summer. The overall value was about 25μg./g. This is in agreement with the figures quoted by the other authors with the exception of Conner & Straub(110), whose value of 59μg./g. is possibly a misprint. Values for buttermilk(126,260) are again higher than for other milk products. The explanation of the phenomenon was given on p. 222. The preparation of riboflavin from whey powder is discussed in (324).

Values for pantothenic acid in whey are given in (159,160,161,163) and for buttermilk in (160,161,163).

The cholic acid-like substance described on p. 222 is absent from dried whey and dried milk(175). A determination of choline hydrochloride gave a value of 0·7 mg./g.(174). The effect of dried whey and other milk products on the stability of carotene and vitamin D has been studied(325,326,327,328).

### (*b*) *Dried and evaporated milk*

#### (i) *General*

A slightly optimistic review of the nutritive value of evaporated milk is given in (329).

Further work on the addition of dry skim milk to flour in bread-making has underlined the marked nutritional benefits of this procedure(322,330) resulting not only from increased calcium content but from the valuable addition of riboflavin and of other members of the vitamin $B_2$ complex. Henry & Kon(317) have demonstrated a marked supplementary relationship between the proteins of dried skim milk and those of the potato when these two products were fed together, but not when given separately. The digestion *in vitro* of condensed milk after storage in the frozen condition has been studied by Corley & Doan(331).

A report from Mitchell's laboratory(332) confirms the findings of Henry & Kon(37) that the availability of milk calcium for rats is not impaired by drying and that it is not increased by the administration of vitamin C. On the other hand, it has been found that milk calcium is more available than that of cauliflower and broccoli(333,334) and of fresh carrots, fresh lettuce and fresh green string beans(332), and of beets, turnips and celery(334). The calcium content of white bread has been studied by Prouty & Cathcart(335) with reference to the use of milk solids in baking.

Mueller *et al.* have continued their work(37) on the effects of cocoa on the nutritive value of milk. When added to whole-milk powder to the extent of 16·5 % it depressed the digestibility of the milk proteins(336). The depressing effect of cocoa on the growth of rats receiving diets of mineralized whole-milk powder and cane sugar has again been demonstrated(337,338), and it seems likely that the tannic substances of cocoa are at least partly responsible.

#### (ii) *Vitamin* $B_1$

The vitamin $B_1$ content of samples of dried milk has been measured by a number of observers (109,111,113,114,119,120,121,319,339). The last authors(339), especially, have examined by a fluorimetric method thirty-two samples of dry skim milk drawn from thirteen States of the U.S.A. Their mean value, 119 i.u./100 g., agrees closely with Kon's(7) estimate. The extremes were 100 and 138 i.u./g. respectively. The estimates of the other workers, sometimes based on single samples, vary from 80 to 188 i.u./100 g.

Several investigators have confirmed the earlier findings(37) of the damaging effects of commercial evaporation on the vitamin $B_1$ content of milk(7,113,121,122,340). The estimate of loss varies from 23 to 60 % of the original value. Knott(122) noticed the least destruction, but found that further loss took place on storage. One author(113) reported also marked losses in drying. This is not the general experience. In sweetened condensed milk which is exposed to much less drastic heat treatment than evaporated milk the loss of vitamin $B_1$ is quite small(7). Kon & Thompson(341) examined thirteen samples of American evaporated milk obtained in this country in the course of one year. The mean vitamin $B_1$ content was 46·8μg./100 g. with extremes of 30 and 57μg./100 g.

(iii) *Riboflavin*

In addition to the two reports by Kemmerer(128,129) mentioned on p. 221 papers (342,343) deal specifically with methods of assay of riboflavin in dried milk products. Difficulties of biological tests caused by deficiencies of the basal diet are discussed in (342), and factors affecting the accuracy of the microbiological test are dealt with in (344). Papers (110,126,128,129,130,260,339,344,345) give figures for the riboflavin content of dried milk, and of these(339,260) deal with longer series of samples. The mean values and ranges were very similar in both investigations, 20·9 (17–25) and 20·6 (18–29)$\mu$g./g. respectively. The results of the other authors are in good agreement with these figures, though Arnold *et al.*(345) and Wagner *et al.*(130) reported lower concentrations of 16–17$\mu$g./g. This may have been due to incomplete extraction of the vitamin by the first-named authors, but the latter used a rat assay method. All these assays come from the U.S.A. and taken by and large indicate a higher riboflavin content of milk there than in this country (cf.(346)). This is confirmed by direct comparisons of evaporated milks of American and English origin carried out by Kon & Thompson(341). The mean value recalculated on the fresh-milk basis for the thirteen American samples was over 180$\mu$g./100 g. and for four English summer samples 145$\mu$g./100 g. The reason for this difference is not clear, but it may possibly be due to the presence in American herds of larger numbers of Channel Island breeds.

Culton & Bird(347) have studied the value of dried skim milk and dried whey as sources of riboflavin for poultry.

(iv) *Nicotinic acid and other members of the vitamin* $B_2$ *complex*

Values for the nicotinic acid content of dried milk are given in (143,148,149,152,153,154) and for evaporated milk in (152) and in the two compilations (156,157) quoted on p. 222 which also quote figures for dried milk.

Bauernfeind *et al.*(160) and Strong *et al.*(161) deal with the pantothenic acid content of dried milk. Experiments by Mohammad *et al.*(348) have shown that dried whole milk is a poor source of the antigreying and growth-stimulating filtrate factor.

(v) *Vitamin* C

A method for the estimation of both forms of ascorbic acid in processed milks and its application to commercial American samples are described in (349). The total ascorbic acid content of seven samples of different types of powdered milk varied from 1·79 to 10·08 mg./100 g., and that of ten samples of evaporated milk from 0·38 to 2·23 mg./100 ml. Another series of twenty-one samples gave a mean value of 1·32 mg./100 ml. with extremes of 0·62 and 2·76 mg./100 ml. respectively. Ultra-violet irradiation of the milk had no appreciable effect on the vitamin C content. Kon & Thompson's values ((341), cf. p. 229) vary from 1·3 to 3·1 mg./100 g. with a mean of 2·1 mg./100 g. Josephson & Doan(350) studied the factors controlling the vitamin C content of evaporated milk. Cho(351) reports figures for vitamin C in Japanese dried milk. The mean value for six samples of full-cream dried milk is 3·95 mg./100 g. Ascorbic acid added to dried milk either as pineapple juice(352) or as the pure compound(353) proved fairly stable. The keeping qualities of dried milk as affected by heat treatment prior to drying and by the addition of ascorbic acid are discussed by Jack & Henderson(354). Snow & Zilva(355) noticed that condensed milk to which synthetic ascorbic acid was added, lost its antiscorbutic activity when mixed with tea and found that the catalytic oxidation was associated with the tannic fraction of tea. Recent observations(7) confirm the original finding of Hume(356) that sweetened condensed milk is a good source of vitamin C.

(vi) *Other factors*

As reported in the previous review(37) evaporation destroys to an appreciable extent the vitamin E and, possibly, vitamin K of milk (9,222) (cf. p. 227). Dried milk is apparently devoid of the 'grass juice' factor(11).

Schuck(357) has expressed the opinion that sodium fluoride is more toxic to rats when dissolved in milk than when mixed with dry rations, but her experiments hardly justify definite conclusions.

# IV. NUTRITIONAL VALUE OF MILK FOR HUMAN BEINGS

## A. General

### (a) *Introduction*

Problems of adequate nutrition, for long of interest to scientists and reformers, have of late been gradually drawn into the sphere of practical politics, a process greatly hastened by the present war. It is to be hoped, and indeed it is likely, that the planned agricultural economy of war time will be continued in some form when war is ended and lead to agricultural policies based on nutritional needs. A vastly increased production of milk and milk products to satisfy the essential demands of children, adolescents and pregnant and lactating women will of necessity form the backbone of any such scheme in peace time.

The importance attached in this country in war time to maximal production of milk and the steps taken to ensure its supply to and increase its consumption by the classes of population entitled to nutritional priority are excellently described in the painstaking study of Denhardt (358). The official report of the National Nutrition Conference for Defense convened by President Roosevelt (359) gives the broad lines of the present American nutritional policy and of impressive plans for the future. An even more recent summary of the activities in this sphere of the United States Department of Agriculture will be found in Stiebeling's paper (360). Much valuable information is given in the *Wartime Nutrition Bulletin* published in this country by the Children's Nutrition Council.

### (b) *Nutritional surveys*

Again results of large numbers of surveys and inquiries have been published, and many of these were connected directly or indirectly with the consumption of milk and milk products; only a brief selection can be quoted here; a number have been ably summarized by Stiebeling & Leverton (361). Information regarding different parts of the United States will be found for example in (362,363,364,365), milk consumption in the U.S. Army and Navy is given in (366,367), while papers (368,369,370,371) deal with conditions in Canada and paper (372) with those in New Zealand. The very low consumption of milk in Puerto Rico is evident from (373). In this country the progress in 1938 of the Milk in Schools Scheme is discussed in (374) and some very recent figures are given in (375). Surveys of milk consumption in various areas in England and Wales just before the war are given in (376). It is noteworthy that of the 11,489 households studied the expenditure on food of 1803, or 16 %, including 39 % of the children, was below the minimum considered compatible with proper nutrition. The upward trend of the consumption of liquid milk by middle-class women between 1935 and 1941 is reflected in the observations of Widdowson & Allington (377). Some data for milk consumption at the beginning of the war are given in (378). The relatively high consumption of milk in the highlands and islands of Scotland is evident from the study of Cathcart *et al.* (379), and an inquiry by Corry Mann (380) emphasizes the large intake in peace time of milk and dairy products by public school boys.

### (c) *General effects*

No large-scale experiments on the value of milk in the diet have been reported, but several smaller inquiries have yielded valuable information. Of special interest is a discussion by Corry Mann (380) of his early experiments, carried out about 1910 (including results not published before), on the addition of milk to the diet of children in poor circumstances living in homes. In one comparison twenty children receiving the basal diet alone gained in 12 months 3·75 lb. in weight and $1\frac{1}{2}$ in. in height, while the corresponding gains for twenty children who received a daily addition of 1 pt. of raw milk was 5·5 lb. and $2\frac{1}{8}$ in. respectively. At that time the bacteriological quality of the milk was very low and there were many instances of milk-borne infection among the children receiving the raw milk. In another similar experiment twenty control children gained 3·65 lb. and $1\frac{1}{2}$ in. respectively, but twenty children given 1 pt. of flash-pasteurized milk showed increments of 5·75 lb. and $2\frac{1}{4}$ in. No infective disturbances were observed on this occasion. Some figures have been given (374) suggesting that children in an East London dining centre who received a modified Oslo meal plus milk did better than children who had milk and an ordinary

hot two-course dinner. In addition to the $\frac{2}{3}$ pt. of milk, the Oslo meal consisted of a salad, $1\frac{1}{2}$ oz. cheese, 3 oz. brown bread, $\frac{3}{4}$ oz. butter and a raw apple.

An important inquiry into the influence of the prenatal diet on mother and child has been reported from Toronto (381,382). From among expectant mothers attending the University Clinic, whose income was low and the diet inadequate, alternate women were selected and given a daily addition of 30 oz. milk, one egg and one orange, 2000 i.u. of vitamin D as 'viosterol' and an iron preparation. Once a week a tin of tomatoes and $\frac{1}{2}$ lb. of Cheddar cheese per person were distributed. A third group of women whose income was higher and who could afford a better diet were advised to increase their intake of milk, eggs, meat and cheese. The mothers receiving the improved diets enjoyed better health in pregnancy and had more normal labour, while their babies had less illnesses up to the age of 6 months than those of the control group. The scale of this experiment was not large, and its planning and conclusions have come under severe criticism (383,384), yet it should be remembered that it does not stand isolated but forms an additional link in an already long chain of evidence of the serious effects of faulty nutrition, their correction by proper diet and of the value of milk in this respect.

The addition of dried skim milk to the diet of pre-school Negro boys from poor families in the District of Columbia, U.S.A., resulted in somewhat greater height gains of treated children over controls in the 4-year-old group, but there was no difference for 3-year-olds (385).

A further report has appeared (386) on the work of the National Birthday Trust Fund, described in the previous review (37).

de Haas & Meulemans (387) have written a useful booklet on the practical aspects of milk as a food for infants in the tropics. From the same laboratory comes a report on the usefulness of lactic acid milk (388), while in this country Mackay (389) has studied the value for infants of mixtures of soya-bean flour with full-cream dried milk.

The passage of milk meals through the intestinal tract of healthy children has been extensively studied by Macy *et al.* (390,391).

Woodward (392) is of the opinion that an excessive intake of milk often leads to loss of appetite in children; in such cases he is inclined to reduce its consumption drastically.

## B. Milk components

### (*a*) *Vitamins*

Further proof of the dangers of sweetened condensed skim milk in infant feeding are given by de Haas *et al.* (393) who found that it was responsible for two-thirds of all cases of xerophthalmia observed in a Batavia hospital in infants up to 2 years of age.

The valuable study of Lane *et al.* (319) disclosed the interesting fact that in the average American diet such as was consumed by the middle two-thirds of the population before enriched bread was introduced, milk and milk products supplied 17·6 % of the vitamin $B_1$, and that milk was, after white bread, the second most important single source of this factor.

The vitamin $B_1$ metabolism of infants who derived this factor from cow's and human milk was studied by Widenbauer & Krüger (394). Wilson (395) noticed in rural children in Oxfordshire several cases of cheilosis of which two were severe and one was in a boy receiving 1 pt. of milk daily. The symptoms were cured by the administration of pure riboflavin. Wilson attributed the riboflavin deficiency to low riboflavin content of milk at certain seasons, but it should be remembered that the magnitude of seasonal variations is not great (cf. pp. 221 and 229).

### (*b*) *Proteins*

Experiments on the utilization of milk nitrogen by adults and children are reported in (273,396,397,398). Fetter (399) found by experiments on hospital patients that the renneting of milk had no effect on the speed of its digestion.

Sydow (400) and Davidson (401) have written on milk allergy. The latter believes that milk is mainly responsible for allergic symptoms of perennial occurrence, but his article seems hardly impartial.

### (c) *Minerals*

During the period under review several laboratories have carried out intensive work on the metabolism of calcium by children and adults, and a number of important papers, most of them dealing with milk, have been published (249,396,397,398,402,403,404,405,406,407,408,409,410,411). Paper (249) is a comprehensive review of many aspects of calcium metabolism by a leading authority on this subject. On the whole, the results have confirmed the findings reported in the previous review (37) that children retain only about 20 % of the ingested calcium (396,397,404,405, 406,407,408). Inorganic calcium was retained as well as that of milk (396,404,406). The utilization of calcium was increased in the presence of lactose (408). Adults utilized milk calcium at least as efficiently as children (402,403,409,410,411). Outhouse *et al.* (410) estimate the requirements for adult maintenance at 10·7 mg. Ca/kg. daily, and Steggerda & Mitchell (411) give a figure of 9·55; they are of the opinion, however, that the body may successfully adapt itself to low calcium regimes though not necessarily with the best results for health. Values quoted by Basu *et al.* (398) for Indians subsisting on low calcium diets give some support to this view (cf. (37)).

Basu & Malakar (412) have studied the magnesium metabolism of man and found that addition of milk to basal diets often caused a decrease in magnesium retention. This they ascribe to the antagonistic effect of milk calcium. The retention of magnesium by infants from milk diets is discussed in (413).

In view of the prevalence of anaemia in women and children on war time diets Mackay *et al.* (414,415) advocate the provision of iron-fortified milk.

## C. Effect of treatment

### (a) *Effect of heating and processing*

Evaporated whole milk is generally accepted as satisfactory in infant feeding (416,417,418,419,420). In the study of Shukers *et al.* (413) different types of processed milk are compared as a source of magnesium for the infant. Steggerda & Mitchell (411) found, in agreement with experiments on rats (cf. p. 229), that commercial drying of milk did not lower the availability of its calcium. The use of buttermilk in infant feeding is discussed by Meulemans & de Haas (421).

### (b) *Irradiated and vitaminized milk*

Information concerning the relative antirachitic value of various sources of vitamin D including fortified milks will be found in the authoritative article by Park (422).

Robinson (423,424) compared the antirachitic effects of irradiated evaporated milk with those of irradiated fresh milks and of irradiated ergosterol added to ordinary evaporated milk or supplementing breast feeding. The results indicate that the therapy was most efficient with breast feeding, otherwise there was no obvious difference between the treatments. In this study the incidence and severity of rickets was much higher than is usually found at present under similar conditions.

Rhoads *et al.* (425) studied the development of children up to 2 years of age receiving diets containing 13 fl. oz. of evaporated milk with various vitamin supplements. They found that 1500 U.S.P. units daily of vitamin D as cod-liver oil afforded no better protection from rickets than 110 units daily as irradiated evaporated milk. However, the children receiving the cod-liver oil made better gains in length. Additions of carotene were without effect on the growth of children receiving irradiated evaporated milk. The vitamin additions did not influence the incidence of infections.

One of the few reports from this country on the value of irradiated evaporated milk in infant feeding comes from Ellis (426). According to him, though such milk of the potency of 165 i.u. per reconstituted quart is a valuable source of vitamin, it should not be relied on as the sole source, especially for premature infants. McMahon (420) reports uniformly satisfactory results from extensive use in infant feeding of a simple formula of irradiated evaporated milk and water without any other additions.

### (c) *Soft-curd milk*

The problem of digestibility of processed milks, especially milks with lowered curd tension, has been reviewed by Chambers (427) in relation to infant feeding. Experiments in which soft-curd milk obtained by sonic or usual homogenization (428) or by treatment with proteolytic enzymes (429) was compared with ordinary boiled or evaporated milk formulae did not disclose any marked differences in the growth or well-being of the infants; it is claimed, however, that the treated milks were easier to feed. Very good results with soft-curd milk prepared by the sonic oscillation method are reported by Bikoff *et al.* (430), and the beneficial effect on the comfort of the infant is again stressed.

S. K. Kon

National Institute for Research in Dairying
University of Reading

## REFERENCES

(1) *Yearb. U.S. Dep. Agric.* (1939).
(2) Roadhouse & Henderson (1941). *The Market Milk Industry.* New York: McGraw-Hill Book Co., Inc.
(3) Davies (1939). *The Chemistry of Milk*, 2nd ed. London: Chapman and Hall.
(4) McCance & Widdowson (1940). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 235.
(5) Chatfield & Adams (1940). *Circ. U.S. Dep. Agric.* no. 549.
(6) Bowes & Church (1940). *Food Values of Portions Commonly Used*, 3rd ed. Philadelphia: Child Health Society.
(7) Kon (1941). *Nature, Lond.*, **148**, 607.
(8) Kennedy (1941). *Bull. Cornell agric. Exp. Sta.* no. 774.
(9) Anderson, Elvehjem & Gonce (1940). *J. Nutrit.* **20**, 433.
(10) Evans & Phillips (1939). *J. Nutrit.* **18**, 353.
(11) Randle, Sober & Kohler (1940). *J. Nutrit.* **20**, 459.
(12) Johnson, Elvehjem & Peterson (1941). *J. Dairy Sci.* **24**, 861.
(13) Anon. (1939–40). *Rep. N. Mex. agric. Exp. Sta.* **51**, p. 59.
(14) Gomez, Hartman & Dryden (1941). *J. Dairy Sci.* **24**, 507.
(15) Hartmen & Cary (1942). *J. Dairy Sci.* **25**, 679.
(16) Rupel, Bohstedt, Wegner & Hart (1940). *Bull. Wis. agric. Exp. Sta.* no. 450, p. 20.
(17) Valenzuela (1940). *Philipp. J. Anim. Ind.* **7**, 453.
(18) Abbott (1940). *Amer. J. publ. Hlth*, **30**, 237.
(19) Bunger (1941).* *Molkereiztg, Hildesh.*, **55**, 945.
(20) Fixsen & Roscoe (1940). *Nutr. Abstr. Rev.* **9**, 795.
(21) Booher, Hartzler & Hewston (1942). *Circ. U.S. Dep. Agric.* no. 638.
(22) Anon. (1940). *J. Milk Tech.* **3**, 232.
(23) Morton (1942). *The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes*, 2nd ed. London: Adam Hilger, Ltd.
(24) Morton, Lord & Goodwin (1941). *J. Soc. Chem. Ind., Lond.*, **60**, 310.
(25) Neal, Haurand & Luckmann (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 150.
(26) Kon (1934). *J. Dairy Res.* **5**, 250.
(27) Koehn (1940). *Rep. Ala. agric. Exp. Sta.* 1939, p. 25.
(28) Koehn (1940). *J. biol. Chem.* **133**, lvi.
(29) Shrewsbury, Kraybill & Zscheile (1939). *Rep. Ind. agric. Exp. Sta.* 1938–9, p. 29.
(30) Thompson (1942). *Chem. & Ind.* **61**, 170.
(31) Garrett, Tucker & Nuissl (1939). *Bull. N.J. agric. Exp. Sta.* no. 671.
(32) Gallup & Kuhlman (1941). *Oil & Soap*, **18**, 71.
(33) Effern (1940). *Vorratspflege u. Lebensmittelforsch.* **3**, 53.
(34) Rosenthal & Erdély (1934). *Biochem. J.* **28**, 41.
(35) Kon (1936). *J. Dairy Res.* **7**, 192.
(36) Kon (1938). *J. Dairy Res.* **9**, 242.

(37) Kon (1940). *J. Dairy Res.* **11**, 196.
(38) Henry, Kon & Thompson (1942). Unpublished data.
(39) Fraps, Kemmerer & Meinke (1941). *J. Ass. off. agric. Chem., Wash.*, **24**, 731.
(40) Shrewsbury & Kraybill (1939). *Rep. Ind. agric. Exp. Sta.* 1938–9, p. 29.
(41) Gillam (1934). *Biochem. J.* **28**, 79.
(42) Johnson, Peterson & Steenbock (1941). *J. Dairy Sci.* **24**, 813.
(43) Johnson & Peterson (1940). *Abstr. Pap. Amer. chem. Soc.* 100*th Mtg*, p. B 16.
(44) Quackenbush, Steenbock & Peterson (1938). *J. Amer. chem. Soc.* **60**, 2937.
(45) Thompson, Kon & Mawson (1942). *Proc. Biochem. Soc.* **36**, xvii.
(46) Gillam & Kon (1940). *J. Dairy Res.* **11**, 266.
(47) Garrett & Cowling (1940). *Guernsey Breed. J.* **57**, 9.
(48) Bartlett, Reece & Cowling (1940). *Proc. Amer. Soc. Anim. Prod.* **39**, 74.
(49) Bartlett, Reece & Cowling (1941). *Guernsey Breed. J.* **59**, 281.
(50) Bartlett, Garrett, Reece & Hartman (1940). *Bull. N.J. agric. Exp. Sta.* no. 675.
(51) Sherman (1941). *J. Nutrit.* **22**, 153.
(52) Hauge, Hilton & Wilbur (1940). *J. Dairy Sci.* **23**, 719.
(53) Williams (1941). *Iowa St. Coll. J. Sci.* **16**, 148.
(54) Tauber (1941). *Ann. Rev. Biochem.* **10**, 47.
(55) Dornbush, Peterson & Olson (1940). *J. Amer. med. Ass.* **114**, 1748.
(56) Booher & Marsh (1941). *Tech. Bull. U.S. Dep. Agric.* no. 802.
(57) Breirem (1939). *Beretn. Nordiske Jordbruksforskeres Forenings Husdjurskongr. i. Falun*, p. 37.
(58) Hutchinson (1940). *Proc. roy. Soc. Vict.* **52** (N.S.), 113.
(59) Julius, Rivett & Richardson (1941). *Rep. Coun. sci. industr. Res. Aust.* 1940–1, p. 74.
(60) Cox (1941). *Rep. Dep. sci. industr. Res. N.Z.* p. 8.
(61) Willstaedt (1941). *Z. Vitaminforsch.* **11**, 340.
(62) Guggenheim (1940). *J. Hyg., Camb.*, **40**, 690.
(63) Ray, Chand & Rau (1941). *J. Dairy Res.* **12**, 109.
(64) Muthanna & Seshan (1941). *Indian med. Gaz.* **76**, 487.
(65) Majumdar (1941). *Indian J. vet. Sci.* **11**, 329.
(66) Doctor & Banerjee (1939). *Curr. Sci.* **8**, 513.
(67) Govindarajan & Banerjee (1940). *Indian J. vet. Sci.* **10**, 335.
(68) Ahmad (1940). *J. Indian med. Ass.* **10**, 1.
(69) Bacharach (1940). *Dairy Industr.* **5**, 209.
(70) Garrett, Arnold & Hartman (1941). *J. Dairy Sci.* **24**, 71.
(71) Swope (1939). *Bull. Pa agric. Exp. Sta.* no. 382.
(72) Bechdel, Stone, Williams & Murdock (1941). *Bull. Pa agric. Exp. Sta.* no. 411.
(73) Johnson, Peterson, King & Bohstedt (1940). *Industr. Engng Chem.* (Industr. ed.), **32**, 1622.
(74) Lease & Mitchell (1940). *Bull. S.C. agric. Exp. Sta.* no. 329.
(75) Johnson & Peterson (1940). *J. Dairy Sci.* **23**, 385.
(76) Atkeson, Bechtel, Shaw & Hughes (1940). *Rep. Kans. agric. Exp. Sta.* 1938–40, p. 79.
(77) Anon. (1939). *Beretn. Forsøgsm., Kbh.*, no. 23, 68 (Aarsberetn. 1938–9).
(78) Deuel & Nuttall (1941). *Cert. Milk*, **16**, No. 186, p. 3.
(79) Deuel, Halliday, Hallman, Johnston & Miller (1941). *J. biol. Chem.* **139**, 479.
(80) Deuel, Halliday, Hallman, Johnston & Miller (1941). *J. Nutrit.* **22**, 303.
(81) Deuel, Hallman, Johnston & Mattson (1942). *J. Nutrit.* **23**, 567.
(82) Blaxter, Kon & Thompson (1942). Unpublished data.
(83) Martin, Cave, Whitnah, Beck & Atkeson (1940). *Ass. Bull.* (*Int. Ass. Milk Dlrs*), **32**, 279.
(84) Rusoff, Skipper & Arnold (1942). *J. Dairy Sci.* **25**, 807.
(85) Wilbur, Hilton & Hauge (1940). *J. Dairy Sci.* **23**, 765.
(86) Sherman (1937). *Chemistry of Food and Nutrition*, 5th ed., p. 357. New York: Macmillan Co.
(87) Wilbur, Hilton & Hauge (1940). *J. Dairy Sci.* **23**, 575.
(88) Rupel, Phillips, Boyer, Lundquist & Lardy (1941). *Bull. Wis. agric. Exp. Sta.* no. 453, p. 14.
(89) Wilson (1942). *Cert. Milk*, **17**, No. 197, p. 5.
(90) Hilton, Wilbur, Westfall & Hauge (1942). *J. Dairy Sci.* **25**, 696.
(91) Copeland & Fraps (1940). *Rep. Tex. agric. Exp. Sta.* 1939, p. 110.
(92) Brown & Thurston (1940). *J. Dairy Sci.* **23**, 629.
(93) Brown, VanLandingham & Weakley (1941). *J. Dairy Sci.* **24**, 925.

(94) Williams & Burgwald (1941). *J. Dairy Sci.* **24**, 539.
(95) Miller, Lease & Anderson (1940). *J. Dairy Sci.* **23**, 573.
(96) Kon & Thompson (1942). Unpublished data.
(97) Richter (1938). *Kühn Arch.* **49**, 269.
(98) Phillips, Lundquist & Boyer (1941). *J. Dairy Sci.* **24**, 977.
(99) Stewart & McCallum (1942). *J. Dairy Res.* **13**, 1.
(100) Harrelson, Nelson, Lowe & Nelson (1939). *Iowa St. Coll. J. Sci.* **13**, 353.
(101) Dyme, Nelson, Lowe & Nelson (1941). *Iowa St. Coll. J. Sci.* **15**, 189.
(102) Gray & Stone (1939).* *Food Industr.* **11**, 626.
(103) Krukovsky (1942). *J. Dairy Sci.* **25**, 297.
(104) Drummond (1940). *Nature, Lond.*, **145**, 53.
(105) Edisbury (1940). *Analyst*, **65**, 484.
(106) Sydenstricker, Singal, Briggs, De Vaughn & Isbell (1942). *J. Amer. med. Ass.* **118**, 1199.
(107) Williams (1942). *J. Amer. med. Ass.* **119**, 1.
(108) Various authors. *Univ. Texas Publ.* No. 4137 (1941).
(109) Conner & Straub (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 380.
(110) Conner & Straub (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 385.
(111) Harris & Wang (1941). *Biochem. J.* **35**, 1050.
(112) Wang & Harris (1942). *Chem. & Ind.* **61**, 27.
(113) Kendall (1942). *J. Pediat.* **20**, 65.
(114) Slater (1941). *Aust. J. exp. Biol. med. Sci.* **19**, 29.
(115) Bertagni (1939). *Boll. Soc. ital. Biol. sper.* **14**, 244.
(116) Prebluda & McCollum (1939). *J. biol. Chem.* **127**, 495.
(117) Halliday & Deuel (1941). *J. biol. Chem.* **140**, 555.
(118) Sakurai (1941). *Abstr. Bull. Inst. phys. chem. Res. Japan*, **20**, 18.
(119) Williams, McMahan & Eakin (1941). *Univ. Texas Publ.* no. 4137, p. 31.
(120) Schultz, Atkin & Frey (1942). *Industr. Engng Chem.* (Anal. ed.), **14**, 35.
(121) Booher & Hartzler (1939). *Tech. Bull. U.S. Dep. Agric.* no. 707.
(122) Knott (1942). *Amer. J. publ. Hlth*, **32**, 1013.
(123) Morgan & Haynes (1939). *J. Nutrit.* **18**, 105.
(124) Sharp & Hand (1940). *Proc. 1st Food Conf. Inst. Food. Tech.* p. 139.
(125) Leviton (1942). *Abstr. Pap. Amer. chem. Soc. 103rd Mtg*, p. B 2.
(126) Chapman & McFarlane (1942). *Canad. J. Res.* **20**, sect. B, 82.
(127) Najjar (1941). *J. biol. Chem.* **141**, 355.
(128) Kemmerer (1940). *J. Ass. off. agric. Chem. Wash.* **23**, 346.
(129) Kemmerer (1941). *J. Ass. off. agric. Chem. Wash.* **24**, 413.
(130) Wagner, Axelrod, Lipton & Elvehjem (1940). *J. biol. Chem.* **136**, 357.
(131) Munsell (1942). *Food Res.* **7**, 85.
(132) Emmerie (1938). *Z. Vitaminforsch.* **7**, 244.
(133) Baars (1938). *Geneesk. Tijdschr. Ned.-Ind.* **78**, 3145.
(134) Sumi & Tsuzuki (1938). *Abstr. Bull. Inst. phys. chem. Res. Japan*, **17**, 64.
(135) Johnson, Maynard & Loosli (1941). *J. Dairy Sci.* **24**, 57.
(136) McElroy & Goss (1940). *J. Nutrit.* **20**, 527.
(137) Hunt, Kick, Burroughs, Bethke, Schalk & Gerlaugh (1941). *J. Nutrit.* **21**, 85.
(138) Wegner, Booth, Elvehjem & Hart (1940). *Proc. Soc. exp. Biol., N.Y.*, **45**, 769.
(139) Wegner, Booth, Elvehjem & Hart (1941). *Proc. Soc. exp. Biol., N.Y.*, **47**, 90.
(140) McElroy & Goss (1941). *J. Nutrit.* **21**, 163.
(141) Sharp & Hand (1941). *Rep. Cornell agric. Exp. Sta.* 1940, p. 121.
(142) Bailey, Dann, Satterfield & Grinnells (1941). *J. Dairy Sci.* **24**, 1047.
(143) Noll & Jensen (1941). *J. biol. Chem.* **140**, 755.
(144) Melnick & Field (1940). *J. biol. Chem.* **134**, 1.
(145) Melnick & Field (1940). *J. biol. Chem.* **135**, 53.
(146) Kodicek (1940). *Biochem. J.* **34**, 724.
(147) Swaminathan (1941). *Indian J. med. Res.* **29**, 325.
(148) Daroga (1941). *J. Soc. chem. Ind., Lond.*, **60**, 263.
(149) Brown, Thomas & Bina (1942). *Cereal Chem.* **19**, 447.
(150) Del Regno (1939). *Quad. Nutriz.* **6**, 368.

(151) Snell & Wright (1941). *J. biol. Chem.* **139**, 675.
(152) Teply, Strong & Elvehjem (1942). *J. Nutrit.* **23**, 417.
(153) Cheldelin & Williams (1942). *Industr. Engng Chem.* (Anal. ed.), **14**, 671.
(154) Waisman, Mickelsen, McKibbin & Elvehjem (1940). *J. Nutrit.* **19**, 483.
(155) Schaefer, McKibbin & Elvehjem (1942). *J. biol. Chem.* **144**, 679.
(156) Bacharach (1941). *Nutr. Abstr. Rev.* **10**, 459.
(157) McVicar & Berryman (1942). *J. Nutrit.* **24**, 235.
(158) Pelczar & Potter (1941). *J. biol. Chem.* **139**, 111.
(159) Pennington, Snell & Williams (1940). *J. biol. Chem.* **135**, 213.
(160) Bauernfeind, Norris & Heuser (1942). *Poult. Sci.* **21**, 136.
(161) Strong, Feeney & Earle (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 566.
(162) McElroy & Goss (1941). *J. Nutrit.* **21**, 405.
(163) Jukes (1941). *J. Nutrit.* **21**, 193.
(164) Swaminathan (1940). *Nature, Lond.*, **145**, 780.
(165) Swaminathan (1940). *Indian J. med. Res.* **28**, 427.
(166) Henderson, Waisman & Elvehjem (1941). *J. Nutrit.* **21**, 589.
(167) Henry & Kon (1942). Unpublished data.
(168) McElroy & Goss (1940). *J. Nutrit.* **20**, 541.
(169) Lampen, Bahler & Peterson (1942). *J. Nutrit.* **23**, 11.
(170) Nielsen & Hartelius (1942). *Biochem. Z.* **311**, 317.
(171) Shull, Hutchings & Peterson (1942). *J. biol. Chem.* **142**, 913.
(172) Melville, Hofmann, Hague & du Vigneaud (1942). *J. biol. Chem.* **142**, 615.
(173) Woolley (1941). *J. biol. Chem.* **140**, 453.
(174) Rhian (1941). *Bull. Wash. agric. Exp. Sta.* no. 410, p. 33.
(175) Almquist, Mecchi & Kratzer (1941). *Proc. Soc. exp. Biol., N.Y.*, **47**, 525.
(176) Guigoz (1940). *Lait*, **20**, 279.
(177) Holmes, Tripp, Woelffer & Satterfield (1940). *Food Res.* **5**, 263.
(178) Gunsalus & Hand (1941). *J. biol. Chem.* **141**, 853.
(179) Woessner, Weckel & Schuette (1940). *J. Dairy Sci.* **23**, 1131.
(180) Holtz (1940). *Hoppe-Seyl. Z.* **263**, 187.
(181) Wachholder & Okrent (1940).* *Hoppe-Seyl. Z.* **264**, 254.
(182) Mendive & Repetto (1940). *Rev. Inst. bact., B. Aires*, **9**, 487.
(183) Lojander (1939). *Acta Soc. Med.* '*Duodecim*', Ser. B, **27**, 1.
(184) Sharp, Hand & Guthrie (1942). *Ass. Bull.* (*Int. Ass. Milk Dlrs*), **34**, 365.
(185) Holmes, Tripp, Woelffer & Satterfield (1940). *Amer. J. Dis. Child.* **60**, 1025.
(186) Holmes, Tripp, Woelffer & Satterfield (1942). *Food Res.* **7**, 370.
(187) Holmes, Tripp, Woelffer & Satterfield (1941). *J. Nutrit.* **22**, 267.
(188) Holmes, Tripp, Woelffer & Satterfield (1942). *Food Res.* **7**, 111.
(189) Wallis (1940). *J. Dairy Sci.* **23**, 569.
(190) Wallis (1942). *J. Dairy Sci.* **25**, 687.
(191) Knight, Dutcher, Guerrant & Bechdel (1940). *Proc. Soc. exp. Biol., N.Y.*, **44**, 90.
(192) Knight, Dutcher, Guerrant & Bechdel (1941). *J. Dairy Sci.* **24**, 567.
(193) Guthrie (1940). *J. Dairy Sci.* **23**, 501.
(194) Brown, Vanlandingham & Weakley (1941). *J. Dairy Sci.* **24**, 1035.
(195) Rasmussen, Guerrant, Shaw, Welch & Bechdel (1936). *J. Nutrit.* **11**, 425.
(196) Kon & Watson (1937). *Biochem. J.* **31**, 223.
(197) Reineke, Garrison & Turner (1941). *J. Dairy Sci.* **24**, 41.
(198) Sharp, Hand & Guthrie (1940). *J. Milk. Tech.* **3**, 228.
(199) Sharp, Hand & Guthrie (1941). *Food Industr.* **13**, No. 6, p. 50.
(200) Sharp, Guthrie & Hand (1940). *Rep. N.Y. St. Ass. Dairy and Milk Insp.* **14**, 63.
(201) Noll & Supplee (1941). *J. Dairy Sci.* **24**, 993.
(202) Sharp, Hand & Guthrie (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 593.
(203) Corbett & Tracy (1941). *Food Res.* **6**, 445.
(204) Nelson & Dahle (1940). *Milk Dlr*, **29**, no. 10, p. 41.
(205) Swanson & Sommer (1940). *J. Dairy Sci.* **23**, 597.
(206) Greenbank (1940). *J. Dairy Sci.* **23**, 725.
(207) Gjessing & Trout (1940). *J. Dairy Sci.* **23**, 373.

(208) Henderson (1940). *Ass. Bull.* (*Int. Ass. Milk Dlrs*), **32**, 271.
(209) Dahle (1941). *Milk Pl. Mon.* **30**, no. 9, p. 29.
(210) Reif (1941). *Milchw. Forsch.* **21**, 1.
(211) Garrett (1940). *J. Dairy Sci.* **23**, 496.
(212) Dills & Nelson (1942). *J. Amer. chem. Soc.* **64**, 1616.
(213) Henderson, Foord & Roadhouse (1940). *Food Res.* **5**, 153.
(214) England & Wiedemer (1941). *Milk Dlr*, **31**, no. 3, p. 33.
(215) Roadhouse & Henderson (1941). *J. Dairy Sci.* **24**, 540.
(216) Weckel (1941). *J. Dairy Sci.* **24**, 445.
(217) Gridgeman, Lees & Wilkinson (1940). *Analyst*, **65**, 493.
(218) Henry & Kon (1942). *Biochem. J.* **36**, 456.
(219) Anon. (1941). *Rep. Ariz. agric. Exp. Sta.* 1939–40, p. 78.
(220) Anderson & Elvehjem (1940). *J. biol. Chem.* **134**, 217.
(221) Booth, Henry & Kon (1942). *Biochem. J.* **36**, 445.
(222) Anderson, Elvehjem & Gonce (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 750.
(223) Mason & Bryan (1940). *J. Nutrit.* **20**, 501.
(224) Salomonsen (1940).* *Acta paediatr., Stockh.*, **28**, 1.
(225) Sells, Walker & Owen (1941). *Proc. Soc. exp. Biol., N.Y.*, **47**, 441.
(226) Buruiana (1940). *C.R. Acad. Sci., Paris*, **210**, 721.
(227) Roberts & Christman (1942). *J. biol. Chem.* **145**, 267.
(228) Leonards, Free & Myers (1942). *Abstr. Pap. Amer. chem. Soc.* 103*rd Mtg*, p. B 5.
(229) Nalder (1941). *Univ. Microfilms Publ.* no. 224.
(230) Richter (1941). *Amer. J. Physiol.* **133**, 29.
(231) Mitchell, Cook & Henderson (1940). *Arch. Ophthal., N.Y.*, **24**, 990.
(232) Henderson & Mitchell (1941). *J. Nutrit.* **21**, 115.
(233) Moore, Henderson, Mitchell & Ritchie (1941). *J. Nutrit.* **21**, 125.
(234) Cook & Mitchell (1942). *Fed. Proc. Part* II, **1**, 188.
(235) Yudkin & Greer (1940). *Arch. Ophthal., N.Y.*, **23**, 28.
(236) Salit, Swan & Paul (1942). *Fed. Proc.* Part II, **1**, 132.
(237) Selle (1940). *Arch. Ophthal., N.Y.*, **23**, 369.
(238) Darby & Day (1940). *J. biol. Chem.* **133**, 503.
(239) Patch (1941). *J. Nutrit.* **22**, 365.
(240) Dizikes & Doan (1942). *J. Dairy Sci.* **25**, 37.
(241) Tracy & Corbett (1940). *J. Dairy Sci.* **23**, 289.
(242) Kelly (1939). *Rep. Bur. Dairy Ind. U.S. Dep. Agric.* 1938–9, p. 40.
(243) Archibald & Parsons (1940). *Bull. Mass. agric. Exp. Sta.* no. 369, p. 18.
(244) Archibald & Parsons (1941). *Bull. Mass. agric. Exp. Sta.* no. 378, p. 20.
(245) Erf (1941). *Proc. Soc. exp. Biol., N.Y.*, **46**, 284.
(246) Archibald (1941). *Milk Pl. Mon.* **30**, no. 9, p. 36.
(247) Anon. (1941). *Rep. Ariz. agric. Exp. Sta.* 1939–40, p. 82.
(248) Foley (1941). *Milk Dlr*, **30**, no. 12, p. 39.
(249) Mitchell (1939). *The Dietary Requirement of Calcium and its Significance.* Actualités scientifiques et industrielles, 771. Nutrition xviii. Paris: Hermann et Cie.
(250) Frost, Potter, Elvehjem & Hart (1940). *J. Nutrit.* **19**, 207.
(251) Frost, Elvehjem & Hart (1940). *J. Nutrit.* **19**, 311.
(252) Frost, Elvehjem & Hart (1941). *J. Nutrit.* **21**, 93.
(253) McKibbin, Schaefer, Elvehjem & Hart (1942). *J. biol. Chem.* **145**, 107.
(254) Anon. (1940) *Rep. Ariz. agric. Exp. Sta.* 1938–9, p. 80.
(255) Barnes, Sperling & Maynard (1941). *Proc. Soc. exp. Biol., N.Y.*, **46**, 562.
(256) Boyer, Shaw & Phillips (1942). *J. biol. Chem.* **143**, 417.
(257) Free (1940). *Proc. Soc. exp. Biol., N.Y.*, **44**, 371.
(258) Daniel & Hewston (1942). *Amer. J. Physiol.* **136**, 772.
(259) Macrae, El-Sadr & Sellers (1942). *Biochem. J.* **36**, 460.
(260) Culton & Bird (1941). *Poult. Sci.* **20**, 3.
(261) Cary (1941). *Rep. Bur. Dairy Ind. U.S. Dep. Agric.* 1940–1, p. 29.
(262) Coward, Key & Morgan (1929). *Biochem. J.* **23**, 695.
(263) Griffith & Wade (1940). *J. biol. Chem.* **132**, 627.

(264) Griffith (1941). *J. Nutrit.* **21**, 291.
(265) Mulford & Griffith (1942). *J. Nutrit.* **23**, 91.
(266) Blumberg, Mackenzie & Seligson (1942). *Fed. Proc.* Part II, **1**, 187.
(267) Meeker & Kesten (1940). *Proc. Soc. exp. Biol., N.Y.*, **45**, 543.
(268) Meeker & Kesten (1941).* *Arch. Path.* **31**, 147.
(269) Anderson, Moxon & Bentley (1941). *Abstr. Pap. Amer. chem. Soc.* 101*st Mtg*, p. A 19.
(270) Gortner (1940). *J. Nutrit.* **19**, 105.
(271) Mueller, Kemmerer, Cox & Barnes (1940). *J. biol. Chem.* **134**, 573.
(272) Cox, Mueller & Kemmerer (1941). *Fed. Proc.* Part II, **1**, 106.
(273) Hartmann, Meeker, Perley & McGinnis (1942).* *J. Pediat.* **20**, 308.
(274) Stokstad (1940). *Poult. Sci.* **19**, 42.
(275) Hogan, Powell & Guerrant (1941). *J. biol. Chem.* **137**, 41.
(276) McCay, Maynard, Sperling & Osgood (1941). *J. Nutrit.* **21**, 45.
(277) Council on Foods and Nutrition (1942). *J. Amer. med. Ass.* **119**, 1425.
(278) Schantz, Boutwell, Elvehjem & Hart (1940). *J. Dairy Sci.* **23**, 1201.
(279) Schantz, Boutwell, Elvehjem & Hart (1940). *J. Dairy Sci.* **23**, 1205.
(280) Boutwell, Geyer, Elvehjem & Hart (1941). *J. Dairy Sci.* **24**, 1027.
(281) Gullickson & Fountaine (1939). *J. Dairy Sci.* **22**, 471.
(282) Gullickson & Fountaine (1942). *J. Dairy Sci.* **25**, 117.
(283) Harris & Rosenfeld (1940). *Abstr. Pap. Amer. chem. Soc.* 100*th Mtg*, p. A 5.
(284) Harris & Mosher (1940). *Food Res.* **5**, 177.
(285) Boer & Jansen (1941). *Voeding*, **2**, 204.
(286) Boer & Jansen (1941). *Voeding*, **3**, no. 2, 15 June.
(287) Euler, Euler & Säberg (1942). *Ernährung*, **7**, 65.
(288) Hagnell-Zethræus, Neymark & Widmark (1939). *K. fysiogr. Sällsk. Handl.* (N.F.), **50**, no. 9.
(289) Schneider, Ascham, Platz & Steenbock (1939). *J. Nutrit.* **18**, 99.
(290) Schneider (1940). *Proc. Soc. exp. Biol., N.Y.*, **44**, 266.
(291) Wilson (1942). *The Pasteurization of Milk.* London: Edward Arnold and Co.
(292) Elvehjem (1941). *Milk Pl. Mon.* **30**, No. 7, p. 26.
(293) Kirsch (1942). *ForschDienst.* **13**, 181.
(294) Hartman & Garrett (1942). *J. Milk Tech.* **5**, 86.
(295) Millenky & Brueckner (1941). *Bull. Cornell agric. Exp. Sta.* no. 754.
(296) Fleisch & Domenjoz (1940). *Z. Vitaminforsch.* **10**, 244.
(297) Fleisch (1942). *Z. Vitaminforsch.* **12**, 86.
(298) Bahrs & Wulzen (1941). *Cert. Milk*, **16**, No. 180, p. 5.
(299) Wulzen & Bahrs (1941). *Amer. J. Physiol.* **133**, p. 500.
(300) McCollum (1942). *Amer. J. publ. Hlth*, **32**, 80.
(301) Bing (1942). *Fed. Proc.* **1**, 336.
(302) Weckel (1940). *Proc.* 1*st Food Conf. Inst. Food. Tech.* p. 133.
(303) Weckel (1941). *Food Industr.* **13**, No. 6, p. 43.
(304) Frey (1941). *Food Industr.* **13**, No. 6, p. 46.
(305) Post (1941). *Food Industr.* **13**, No. 6, p. 41.
(306) Beck, Weckel & Jackson (1940). *Bull. Wis. agric. Exp. Sta.* no. 450, p. 5.
(307) Anon. (1941). *Milchw. Zbl.* **70**, 17.
(308) Vieilly (1940). *Lait*, **20**, 517.
(309) Oser (1942). *Fed. Proc.* **1**, 343.
(310) Russell (1940). *J. Ass. off. agric. Chem., Wash.*, **23**, 341.
(311) Russell (1941). *J. Ass. off. agric. Chem., Wash.*, **24**, 403.
(312) Peterson & Dornbush (1940). *Bull. Wis. agric. Exp. Sta.* no. 450, p. 25.
(313) Schroeder (1939). *Münch. med. Wschr.* **86**, 821.
(314) Krauss, Sutton, Burgwald, Washburn & Bethke (1941). *J. Dairy Sci.* **24**, 691.
(315) Lane (1941). *Milk Pl. Mon.* **30**, No. 5, p. 44.
(316) Sidney Wanzer & Sons (1942). *Milk Pl. Mon.* **31**, No. 3, p. 30.
(317) Henry & Kon (1942). *Chem. & Ind.* **61**, 97.
(318) Kay (1941). *Chem. & Ind.* **60**, 411.
(319) Lane, Johnson & Williams (1942). *J. Nutrit.* **23**, 613.
(320) Rogers (1939). *Rep. Bur. Dairy Ind. U.S. Dep. Agric.* p. 32.

(321) Callaghan & McDonald (1940). *J. Dep. Agric. S. Aust.* **43**, 584.
(322) Henry, Houston, Kon, Powell, Carter & Halton (1940). *J. Dairy Res.* **12**, 184.
(323) Sullivan & Bloom (1940). *J. Milk Tech.* **3**, 346.
(324) Wyatt (1939). *Iowa St. Coll. J. Sci.* **14**, 103.
(325) Fritz, Archer & Barker (1942). *Poult. Sci.* **21**, 361.
(326) Fritz, Halpin, Hooper & Kramke (1942). *Industr. Engng Chem.* (Industr. ed.), **34**, 979.
(327) Peterson, Hughes & Scott (1941). *Abstr. Pap. Amer. chem. Soc.* 101*st Mtg*, p. A 8.
(328) Fraps, Meinke, Reiser & Kemmerer (1941). *Abstr. Pap. Amer. chem. Soc.* 101*st Mtg*, p. A 8.
(329) Eichelberger (1940). *Amer. J. publ. Hlth*, **30**, 169.
(330) Mitchell, Hamilton & Shields (1942). *Milk Pl. Mon.* **31**, No. 6, p. 24.
(331) Corley & Doan (1940). *Food Res.* **5**, 369.
(332) Shields, Fairbanks, Berryman & Mitchell (1940). *J. Nutrit.* **20**, 263.
(333) Fincke (1941). *J. Nutrit.* **22**, 477.
(334) Shields & Mitchell (1941). *J. biol. Chem.* **140**, cxv.
(335) Prouty & Cathcart (1939). *J. Nutrit.* **18**, 217.
(336) Lipman & Mueller (1940). *J. Dairy Sci.* **23**, 524.
(337) Mueller (1942). *J. Dairy Sci.* **25**, 221.
(338) Kinder, Mueller & Mitchell (1942). *J. Dairy Sci.* **25**, 401.
(339) O'Malley & Baldi (1942). *J. Milk Tech.* **5**, 138.
(340) Daniels (1941). *Amer. J. Dis. Child.* **62**, 127.
(341) Kon & Thompson (1942). Unpublished data.
(342) Sullivan, Beaty, Bloom & Reeves (1940). *Abstr. Pap. Amer. chem. Soc.* 100*th Mtg*, p. B 14.
(343) Anon. (1941). *J. Ass. off. agric. Chem., Wash.* **24**, 86.
(344) Bauernfeind, Sotier & Boruff (1942). *Industr. Engng Chem.* (Anal. ed.), **14**, 666.
(345) Arnold, Lipsius & Greene (1941). *Food Res.* **6**, 39.
(346) Houston, Kon & Thompson (1940). *J. Dairy Res.* **11**, 145.
(347) Culton & Bird (1940). *Poult. Sci.* **19**, 424.
(348) Mohammad, Emerson, Emerson & Evans (1940). *J. biol. Chem.* **133**, 17.
(349) Woessner, Elvehjem & Schuette (1940). *J. Nutrit.* **20**, 327.
(350) Josephson & Doan (1942). *J. Dairy Sci.* **25**, 719.
(351) Cho (1939). *Mitt. med. Akad. Kioto*, **26**, 1033.
(352) Yamamoto & Haha (1940).* *J. agric. chem. Soc. Japan*, **16**, 381.
(353) Falke & Lemmel (1939). *Ernährung*, **4**, 317.
(354) Jack & Henderson (1942).* *Food Industr.* **14**, No. 3, p. 50.
(355) Snow & Zilva (1942). *Biochem. J.* **36**, 641.
(356) Hume (1921). *Biochem. J.* **15**, 163.
(357) Schuck (1939). *J. dent. Res.* **18**, 491.
(358) Denhardt (1942). *Food Control in Great Britain.* Intern. Lab. Office Studies and Reports, Series B, no. 35. Montreal: I.L.O.
(359) *Proceedings of the National Nutrition Conference for Defense* (1942). Washington, D.C.: Federal Security Agency.
(360) Stiebeling (1942). *Fed. Proc.* **1**, 327.
(361) Stiebeling & Leverton (1941). *Ann. Rev. Biochem.* **10**, 423.
(362) Stiebeling, Monroe, Coons, Phipard & Clark (1941). *Misc. Publ. U.S. Dep. Agric.* no. 405.
(363) Clayton (1940). *Bull. Me agric. Exp. Sta.* no. 401.
(364) Borsook & Halverson (1940). *Amer. J. publ. Hlth*, 30, 895.
(365) Strowd (1942). *Abstr. Pap. Amer. chem. Soc.* 103*rd Mtg*, p. A 22.
(366) Howe (1942). *J. Amer. med. Ass.* **120**, 93.
(367) Brown (1942). *J. Amer. med. Ass.* **120**, 96.
(368) Patterson & McHenry (1941). *Canad. publ. Hlth J.* **32**, 251.
(369) Young (1941). *Canad. publ. Hlth J.* **32**, 236.
(370) Sylvestre & Nadeau (1941). *Canad. publ. Hlth J.* **33**, 241.
(371) Boucher & Hopper (1939). *Publ. Dep. Agric. Canad.* no. 658 (*Tech. Bull.* no. 22).
(372) Wilson (1939–40).* *Rep. Nutrit. Comm. med. Res. Coun. N.Z.* p. 9.
(373) Cook, Atmayer & Dalman (1940).* *Puerto Rico J. publ. Hlth*, **16**, 3.
(374) Board of Education (1940). *Rep. med. Offr Bd Educ., Lond.*, 1938, p. 23.
(375) Jameson (1942). *Lancet*, **243**, 475.

(376) Murray & Rutherford (1941). *Milk Consumption Habits. Preliminary Report.* Oxford: Agric. Econ. Res. Inst.
(377) Widdowson & Allington (1941). *Lancet*, **241**, 361.
(378) Anon. *Report* (1942). *Lancet*, **242**, 338.
(379) Cathcart, Murray & Beveridge (1940). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 242.
(380) Corry Mann (1939). *J.R. Inst. publ. Hlth*, **2**, 486.
(381) Ebbs, Tisdall & Scott (1941). *J. Nutrit.* **22**, 515.
(382) Ebbs, Brown, Tisdall, Moyle & Bell (1942). *Canad. med. Ass. J.* **46**, 6.
(383) Fisher (1943). *Brit. med. J.* **1**, 144.
(384) Bell (1942). *Proc. R. Soc. Med.* **36**, 65.
(385) Hunt (1939). *Child Develpm.* **10**, 241.
(386) Williams (1939). *Publ. Hlth, Lond.*, **52**, 314.
(387) de Haas & Meulemans (1940). *Melk in het bijzonder als Zuigelingenvoedsel.* Batavia: Drukkerij Smits.
(388) Posthuma & de Haas (1940). *Geneesk. Tijdschr. Ned.-Ind.* **80**, 888.
(389) Mackay (1940). *Arch. Dis. Child.* **15**, 1.
(390) Macy, Reynolds, Souders & Olson (1940). *Amer. J. Roentgenol. and Radium Therapy*, **43**, 394.
(391) Reynolds, Macy, Hunscher & Olson (1940). *Amer. J. Roentgenol. and Radium Therapy*, **43**, 517.
(392) Woodward (1941). *Tex. St. J. Med.* **37**, 297.
(393) de Haas, Posthuma & Meulemans (1940). *Geneesk. Tijdschr. Ned.-Ind.* **80**, 928.
(394) Widenbauer & Krüger (1939). *Z. Kinderheilk.* **61**, 52.
(395) Wilson (1941). *Lancet*, **241**, 405.
(396) Hsu & Adolph (1940). *Chin. J. Physiol.* **15**, 317.
(397) Daniels (1941). *Amer. J. Dis. Child.* **62**, 279.
(398) Basu, Basak & De (1941). *Indian J. med. Res.* **29**, 105.
(399) Fetter (1941). *Rev. Gastroenterol., N.Y.*, **8**, 120.
(400) Sydow (1939).* *Acta paediatr., Stockh.*, **23**, 383.
(401) Davidson (1942). *Sth. med. J. Birmingham*, **35**, 196.
(402) Steggerda (1939). *Manual Dairy Manuf. Conf. Univ. Illinois*, p. 59.
(403) Steggerda (1940). *Amer. J. Physiol.* **129**, p. 471.
(404) Pierce, Daggs, Meservey & Simcox (1940). *J. Nutrit.* **19**, 401.
(405) Outhouse, Mills, Breiter, McKey, Rutherford, Armstrong & Mitchell (1940). *J. Nutrit.* **19**, Suppl. p. 8.
(406) Kempster, Breiter, Mills, McKey, Bernds & Outhouse (1940). *J. Nutrit.* **20**, 279.
(407) Hess, Poncher, Wade & Ricewasser (1940). *Amer. J. Dis. Child.* **60**, 535.
(408) Mills, Breiter, Kempster, McKey, Pickens & Outhouse (1940). *J. Nutrit.* **20**, 467.
(409) Breiter, Mills, Dwight, McKey, Armstrong & Outhouse (1941). *J. Nutrit.* **21**, 351.
(410) Outhouse, Breiter, Rutherford, Dwight, Mills & Armstrong (1941). *J. Nutrit.* **21**, 565.
(411) Steggerda & Mitchell (1941). *J. Nutrit.* **21**, 577.
(412) Basu & Malakar (1940). *Indian J. med. Res.* **28**, 333.
(413) Shukers, Knott & Schlutz (1941). *J. Nutrit.* **22**, 53.
(414) Mackay, Wills, Dobbs & Bingham (1942). *Lancet*, **243**, 32.
(415) Wills, Mackay, Bingham & Dobbs (1942). *J. Hyg., Camb.*, **42**, 505.
(416) Riesenfeld & Lichtenberg (1938).* *Arch. Pediat.* **55**, 553.
(417) Birmele (1939).* *Mschr. Kinderheilk.* **78**, 201.
(418) Fowler (1939).* *Arch. Pediat.* **56**, 535.
(419) Scheer (1939).* *Arch. Kinderheilk.* **117**, 180.
(420) McMahon (1939). *Wis. med. J.* **38**, 874.
(421) Meulemans & de Haas (1940). *Geneesk. Tijdschr. Ned.-Ind.* **80**, 2465.
(422) Park (1940). *J. Amer. med. Ass.* **115**, 370.
(423) Robinson (1940). *Amer. J. Dis. Child.* **59**, 816.
(424) Robinson (1940). *Amer. J. Dis. Child.* **59**, 1002.
(425) Rhoads, Rapoport, Kennedy & Stokes (1941). *J. Pediat.* **19**, 169.
(426) Ellis (1939). *Arch. Dis. Childh.* **14**, 295.
(427) Chambers (1939). *Rep. N.Y. St. Ass. Dairy and Milk Insp.* **13**, 179.
(428) Wolman (1940). *Rep. N.Y. St. Ass. Dairy and Milk Insp.* **14**, 201.
(429) Blatt, Harris, Jacobs & Zeldes (1940). *J. Pediat.* **17**, 435.
(430) Bikoff, Brown, Zukauckas & Regan (1941). *N.Y. St. J. Med.* **41**, 2052.